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ON THE BREEDING HABITS AND DEVELOPMENT OF AN INDIAN CARP, *CIRRHINA MRIGALA* (HAMILTON)

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A reasonably complete knowledge of life-histories and habits of fishes is needed as a basis for the adoption of adequate measures for conservation of our fisheries and for the production of maximum quantity through proper propagation. It is, however, a matter of regret that the breeding habits and development of Indian Carps specially the larger species, such as *Cirrhina mrigala* (Hamilton), *Labeo rohita* (Hamilton), *Catla catla* (Hamilton), and others are very meagrely known. The author published in 1924, a general account of the breeding habits of *Cyprinidæ* and described the early stages in the development of *Labeo gonius* (Hamilton) (Hamid Khan, 1924, 1925). Jones (1938 *a*, *b*; 1941) studied the development of small-sized Cyprinoid species, namely *Danio malabaricus* (Jerdon) and *Garra ceylonensis ceylonensis* (Bleeker).

Material and Methods

Cirrhina mrigala is one of the commonest carps in India and greatly prized as a food fish. It is widely distributed in the rivers in the Punjab, Bengal, Deccan, Sind and Burma. It grows to about 3 feet in length and attains a weight of 18 pounds or so.

Observations on its breeding habits were carried out at the Departmental Fish Farm, Chhenawan, where the fish spawned on the 25th, 18th and 17th July, in 1928, 1929 and 1933 respectively. On these occasions ripe females were netted from spawning grounds actually in the act of spawning and were stripped. The eggs were obtained in a wash basin and fertilized by mixing them with milt from the male. The excess of milt was washed off after 15 minutes and the fertilized eggs were placed in hatching trays, which were left floating in the tank. The development stages were studied after every five minutes till the completion of segmentation, and then after every half an hour till hatching. After hatching, the study was continued every four hours. Stages were fixed in Bouin's fluid for histological study, while observations on blood circulation were recorded on

live specimens, as in the preserved specimens it becomes impracticable to trace the course of blood. Sketches were drawn with Baker's Drawing Eyepiece or Camera Lucida.

Spawning

Cirrhina mrigala, like most of the carps, breeds in July during monsoon rains. The fish become ripe in May and wait for the first heavy flood to lay their eggs. If the floods are insufficient to inundate their spawning grounds, the fish refuse to spawn and the eggs degenerate within the ovaries. If the floods occur in time the fish leave the main stream, run up the side streams and move on to the inundated fields where water is shallow. Female is often followed by two or three males, and very frequently reverse cases are also seen. On such occasions fish are not shy and can easily be caught with a net or killed with a stick or a stone. In the inundated fields, males and females play together with their bodies pressed and tails slightly bent round each other and splash the water. Many a time it is noticed that their heads are thrust out of water. They remain in this position for a short time with their caudal portion pressed together, and it is at such time that eggs from the female are expelled and immediately fertilized by the male. The eggs are not laid at one place as the fish remain on the move with tremendous splashing and churning of water and go on repeating the process of egg-laying. The actual expulsion of the eggs has not been observed due to turbidity of flood water where the eggs are laid, but the eggs from a female caught in the act of spawning simply flow out of its urino-genital aperture with a slight pressure on its belly. The spawning lasts for a few hours and generally begins during the night or in early hours of the morning and may continue till late in the afternoon. Fertilization is external and the male pours its milt or seminal fluid, which is milky white, non-sticky and non-granular, on the eggs immediately after they are expelled. It has been observed that the milt coagulates within two minutes of its expulsion in water and all the sperms die. Fertilization, in order to be effective, must, therefore, be a speedy process.

Stripping.—The stripping of a ripe female fish either before floods or as soon as it has entered the inundated fields is not practicable as it results in expulsion of hard opaque eggs, which are often mixed with blood and as such cannot be fertilized. But when the fish have indulged in sexual play and are actually spawning, the stripping is easy and the female yields its eggs with a slight pressure. These eggs are soft and translucent and can successfully be fertilized by mixing them with milt from the male. The male, however, yields its milt readily on stripping at all times during the breeding season.

Reproductive powers.—Cirrhina mrigala, like all other carps, possesses an extraordinary fecundity. A ripe female fish weighing 2 lb. has been found to contain 1,24,800 eggs in its ovaries, and another one, weighing 3½ lb. had 2,16,800 eggs (Hamid Khan, 1924).

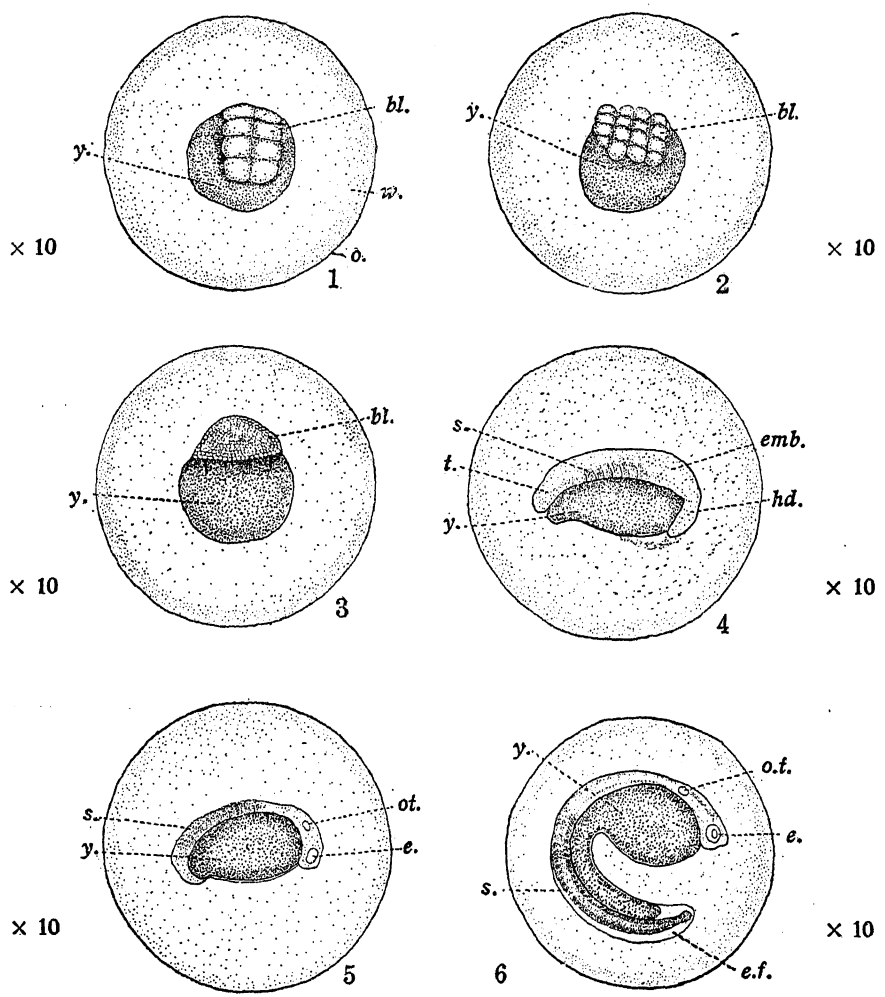
Development

Egg.—When fully ripe, the eggs are spherical in form, almost transparent, yellowish white in colour, non-adhesive, and non-floating. The yolk sphere contains no oil globule. Each measures 1·5 mm. in diameter and is heavily yolked. As soon as it falls into water it begins to swell and within 15 minutes attains a size of 3 to 4 mm. in diameter.

Segmentation.—As soon as fertilization takes place the protoplasm becomes concentrated at one pole of the yolk sphere to form blastodisc. Five minutes after fertilization the first act of cleavage occurs dividing the blastodisc into two cells. Segmentation is partial or discoidal and typically teleostean. After ten minutes four cells are seen, fifteen minute stage has eight cells. Eight blastomeres formed by the third cleavage lie in two symmetrical series of four cells each (Fig. 1). Sixteen blastomeres are seen after 40 minutes (Fig. 2). Blastoderm in advanced stage of cleavage is observed within two hours after fertilization (Fig. 3). During early stages of segmentation blastoderm is a lens-shaped mass of cells and as segmentation advances it becomes dome-shaped and spreads over the yolk. The peripheral margin of the blastoderm is thickened to form the germ ring. As blastoderm grows larger, the germ ring increases in width and advances around the yolk sphere. In three and half hours' stage the blastoderm has covered nearly half of the yolk sphere, and in four and half hours stage, it has spread almost over the whole of the yolk, with a yolk plug, forming the blastopore at one end. Rudiments of embryo appear in seven hours' stage in the form of a belt lying over the yolk sac, which has become elliptical in shape with well differentiated head and tail ends (Fig. 4, *emb.*). By the time the embryo is differentiated the blastoderm has covered the whole of the yolk sphere and the blastopore has closed.

Embryonic development.—Nine hours after fertilization the embryo becomes well defined with seven to eight somites. Segmentation of body advances rapidly and an hour later twelve somites are visible (Fig. 5, *s.*). Eyes (*e.*) appear as transparent objects on the head end. Some of the embryos of ten hours stage have sixteen to seventeen somites with well-developed head, eyes and possess a pair of otocysts with two otoliths (*ot.*) in each, and some of the embryos show movement in the egg shell.

In eleven to twelve hours' stage, there are twenty seven to thirty somites with definite movement of the tail. Yolk sac is prolonged posteriorly up to the tail end. In twelve to fourteen hours stage (Fig. 6), tail is much elongated and projects beyond the yolk sac, and its movements are quick. There are thirty-two somites (*s.*). Eyes (*e.*) are without pigment. Notochord is cellular. Embryonic fin-fold is visible (*e.f.*). In fifteen to sixteen hours'



TEXT-FIGS. 1–6. Development stages of *Cirrhina mrigala*.—Fig. 1. Eight-celled stage, fifteen minutes after fertilization. Fig. 2. Sixteen-celled stage, forty minutes after fertilization. Fig. 3. Two hours' stage. Fig. 4. Seven hours' stage with seven somites. Fig. 5. Ten hours' stage with 15 somites. Fig. 6. Fourteen hours' stage with 32 somites. *bl.*, blastoderm; *e.*, eye; *e.f.*, embryonic finfold; *emb.*, embryo; *hd.*, head; *o.*, outer egg membrane; *o.t.*, otocyst; *s.*, somite; *t.*, tail end of embryo; *w.*, space filled with water; *y.*, yolk.

stage heart has appeared as a simple tabular structure in all the embryos. Auditory and optic vesicles are visible.

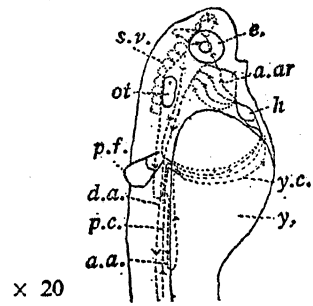
Hatching started at sixteen hours and continued up to nineteen hours. The hatching of a egg lying in a petri-dish was observed under the binocular. The tail ruptured the egg shell and the embryo wriggled out, tail first and came to the surface of water by swirling movement.

Newly hatched embryo (Fig. 7).—The length of newly hatched embryo ranges from 3·8 to 4 mm. The embryo is colourless without any pigment and almost transparent. It moves with a side to side or rotatory movement. Eyes (*e.*) are round and without pigment. Auditory and optic vesicles are visible. Otocysts (*o.t.*), with two otoliths in each, are present. Notochord is cellular. Heart (*h.*) pulsates with dorsal aorta (*d.a.*) which turns back at the caudal end to form the caudal vessel (*c.v.*) which in its turn runs forward to enter the yolk mass and breaks up into capillaries (*y.c.*). The yolk capillaries join together and enter the heart. Tail has become elongated. Yolk sac (*y.*) is prolonged posteriorly and the future anal aperture is marked by a slight depression near the posterior end of the yolk sac. The mouth is not open. Gills have not yet appeared. Embryonic fin-fold (*e.f.*) is present but fins are not yet formed.

Post-embryonic development.—Seven hours after the hatching, three aortic arches are visible and two hours later, the fourth makes its appearance (Fig. 8, *a.ar.*). Two of these arches send blood to the eye and the brain and two to the dorsal aorta. The blood vessel to the eye turns back over the otocyst, forms a loop and then enters the yolk sac. The caudal vessel (*c.v.*), breaks up into capillaries just behind the posterior end of the yolk sac and is continued anteriorly as a single vessel, which enters the yolk sac to form the yolk capillaries (*y.c.*). Segmental vessels have also appeared in the anterior part of the embryo. Heart pulsates at the rate of 140 beats per second and is tabular.

Eleven hours after hatching, pectoral fins appear as bud-like outgrowths. Five aortic arches and three to four branchial arches are also visible. Four hours later (Fig. 9), pectoral fins are prominently marked (*p.f.*), and the branchial region together with mandibular arch shows movement though mouth is not yet open. There are nine to eleven segmental vessels in the head region up to the otocyst, and two posterior to it (Fig. 9, *s.v.*). Segmental vessels are present in the caudal region as well. A vessel (Fig. 9, *a.a.*) branches from the dorsal aorta (*d.a.*), runs posteriorly, enters the yolk, and then runs anteriorly to join the vitelline vessels. There are five aortic arches (*a.ar.*), two anterior ones send the blood to the head and

the eye, the three posterior ones to the dorsal aorta. Blood circulation in the eye is complicated. Two blood vessels take the blood from the head, flow into the yolk and then pass on to the heart along with the yolk capillaries (Fig. 9, *y.c.*).

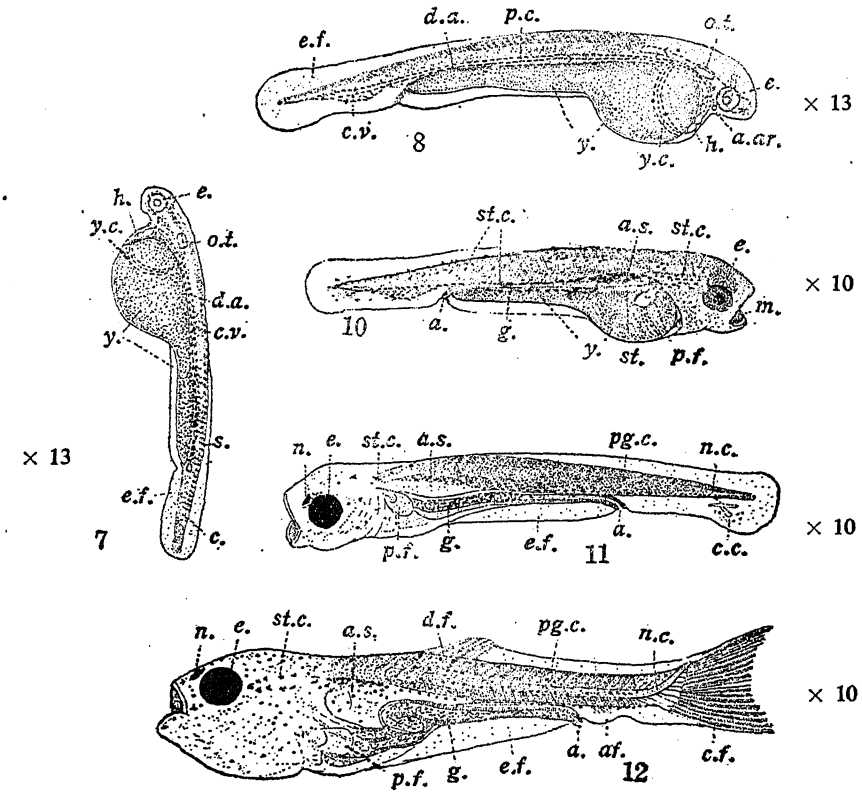


TEXT-FIG. 9. Embryo of *Cirrhina mrigala*, fifteen hours after hatching, showing circulation in the head. *a.a.*, blood vessel branching from dorsal aorta ; *a.ar.*, aortic arches ; *d.a.*, dorsal aorta ; *e.*, eye ; *h.*, heart ; *ot.*, otocyst ; *p.c*, posterior cardinal vein ; *p.f.*, pectoral fin ; *s.v*, segmental vessels ; *y.*, yolk ; *y.c.*, yolk capillaries.

By the end of the first day, *i.e.*, 24 hours after hatching, the pectoral fins are well developed. The embryo has increased in length, measures 5·6 mm. and shows typical teleostean circulation. Six aortic arches on each side function with full strength. Gills have appeared as lobular outgrowths attached to branchial arches and blood vessels pass through them. The blood in the first two aortic arches go direct to the head while in the last four arches it is pumped into the gills, and then to the dorsal aorta. The caudal lacunar circulation behind the future anus is now confined to two vessels with anastomosing branches. Operculum has just appeared but does not as yet extend over the gills. Depressions for the mouth and anus are well marked. Yolk sac is now reduced. There are four to five branchial arches. Air sac has appeared as a space over the yolk. Eyes are slightly pigmented.

Second day.—Embryo measures 5·8 mm. in length (Fig. 10). Mouth (*m.*) is open for respiration. Yolk sac (*y.*) is reduced and has, in addition to yolk capillaries, one vitelline vein at its dorsal region. Eyes (*e.*) have become pigmented, purple with yellow border dorsally. Yellow pigment over the head renders it opaque. Black stellate cells (*st.c.*) have appeared on the ventral and lateral surface of the notochord extending from posterior end of the head to the caudal portion. Air sac (*a.s.*) has extended posteriorly. Stomach (*st.*) is visible under the air sac in the yolk and has a lumen in it, but no lumen is seen in the intestine, which is a prolongation of the stomach and extends posteriorly to the future anus (*a.*). In the yolk there are only

small blood vessels taking blood to the heart. Caudal circulation has extended to the posterior end of the embryo. By the end of the second day,



TEXT-FIGS. 7, 8, 10, 11 and 12. Development of *Cirrhina mrigala*.—Fig. 7. Newly hatched out embryo. Fig. 8. Nine hours stage after hatching. Fig. 10. Second day embryo, 31 hours after hatching. Fig. 11. Four days' old embryo. Fig. 12. Twelve days' old embryo. *a.*, anus; *a.ar.*, aortic arches; *a.s.*, air sac; *c.c.*, caudal capillaries; *c.v.*, caudal vein; *d.a.*, dorsal aorta; *e.*, eye; *e.f.*, embryonic fin; *g.*, gut; *h.*, heart; *m.*, mouth; *n.*, nostril; *n.c.*, notochord; *o.t.*, otocyst; *p.c.*, posterior cardinal vein; *p.f.*, pectoral fin; *pg.c.* pigment cells; *s.*, somite; *st.c.*, stellate cells; *y.*, yolk; *y.c.*, yolk capillaries.

the gills are covered by the operculum. Air sac is filled with gas. Yolk sac is considerably reduced. Gullet is not yet open but lumen is appearing in the intestine. There are no anastomosing capillaries behind the anus. In some specimens two caudal capillaries are seen forming a loop ventral to notochord at the caudal region (Fig. 13, *c.c.*).

Third day.—Length is 6·3 mm. Mouth is open, gut is formed and in some specimens it has food in it, and opens to the exterior. Yolk sac is considerably reduced and lies ventral to the gut. Eyes are brilliantly reddish yellow and purple in colour with golden yellow border round the

pupil. There are two caudal capillary loops and stellate cells have appeared over them. By the end of the day stomach has enlarged. Outlines of vertebræ are visible. Pectoral fin has enlarged. Stellate and round pigment cells have appeared on the head and body. There are four caudal capillaries with radially arranged stellate cells. The fry are very active.

Fourth day.—(Fig. 11). Length of the fry has not increased. Yolk sac is completely absorbed. Caudal fin rays have appeared (Fig. 14, *c.r.*), and by the end of the day, six to seven rays are visible and the caudal capillary circulation has increased.

Fifth day.—Length 6·65 mm. Large stellate cells (Fig. 15, *st.c.*) are arranged laterally on the body along the ventral edge of notochord and dorsal border of body segments. There are six to seven caudal rays and four distinct and two indistinct basal cartilages (Fig. 15, *c.r.*, *b.c.*). Notochord is slightly bent at its posterior extremity. By the end of the day some of the specimens have twelve distinct caudal rays and six basal cartilages.

Sixth day.—Length has not increased. Notochord is well bent upwards and there are fourteen to sixteen caudal rays with six basal cartilages. Internally, liver is visible as a red spot near the air sac. Gut is full of food. Heart consists of three pulsating chambers and receives anterior and posterior vena cavæ, and one blood vessel from the stomach which breaks into capillaries in the liver region and then enters the heart.

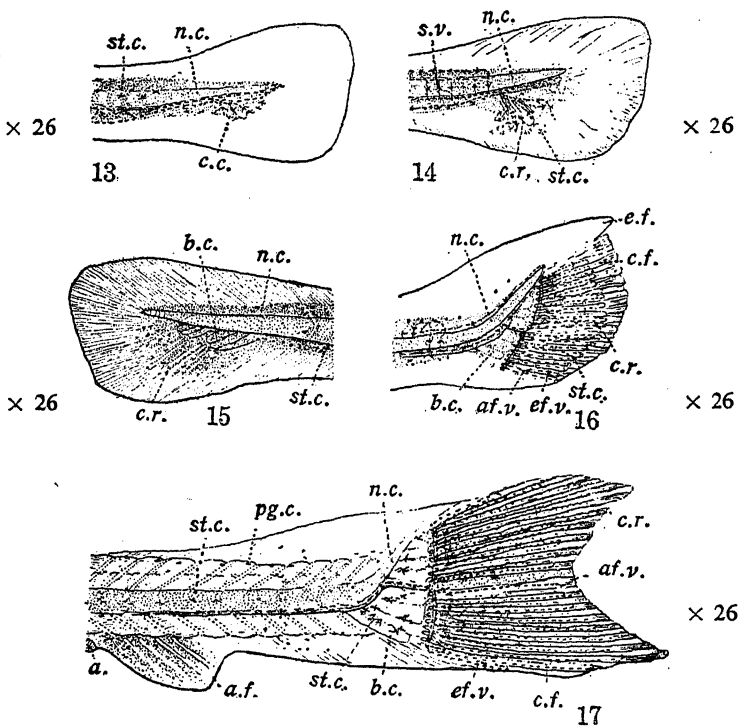
Seventh day.—Owing to curvature of body length has not increased. Caudal rays number seventeen to twenty. In specimens with seventeen to eighteen caudal rays, dorsal fin is appearing as an elevated ridge dorsally with one capillary vessel. In specimens with nineteen to twenty caudal rays dorsal fin has four fin rays and many capillaries.

Eighth day.—Length 7 mm. Structure practically the same as on the seventh day. Caudal fin has ten rays with three basal cartilages in its dorsal half and nine caudal rays and three basal cartilages on its ventral half. There are round pigment cells scattered over the body and stellate cells around the caudal rays and the notochord. Fry reared in small and large live cars showed considerable difference in their growth. Those reared in large live car, measuring 4′ × 3′ × 3′, had eighteen to twenty caudal rays, while those in small live car, measuring 2′ × 1′ × 1′, were under-developed and had six to ten caudal rays.

In 1928 fry were attacked by a protozoan ectoparasite belonging to Cychlochætæ, which infested the embryonic fin-fold, adhered to the gills

and the fry died in large number. Salt bath of 1% strength for 10 minutes cured many of the fry in early stage of the attack. On the 9th and 10th day, structure was almost the same as on the 8th day.

Eleventh day.—Length from 7 to 7·3 mm. Stellate cells round the caudal rays have become radially arranged (Fig. 16, *st.c.*). Caudal rays are 19 to 20 in number and are jointed. Notochord is bent dorsally. Dorsal fin has 4 to 6 rays. In the kacha pond, where weed had been planted to serve as food and also for protection, the fry showed extraordinarily rapid growth. These fry on the eleventh day measured 8 to 9·5 mm. in length and had fully developed dorsal fin with fifteen rays and well-formed caudal, and pectoral fins. It indicates the difference in rearing fry in confinement and under natural conditions.



TEXT-FIGS. 13–17. Development of caudal fin of *Cirrhina mrigala*.—Fig. 13. Caudal fin of second day embryo. Fig. 14. Caudal fin of four days' old embryo. Fig. 15. Caudal fin of five days' old embryo. Fig. 16. Caudal fin of eleven days' old embryo. Fig. 17. Caudal fin of seventeen days' old embryo. *a.*, anus ; *a.f.*, anal fin ; *af.v.*, afferent vessel ; *b.c.*, basal cartilage ; *c.c.*, caudal capillaries ; *c.f.*, caudal fin ; *c.r.*, caudal fin ray ; *d.f.*, dorsal fin ; *e.f.*, embryonic fin ; *ef.v.*, efferent vessel ; *n.c.*, notochord ; *pg.c.*, pigment cell ; *st.*, stellate cells ; *s.v.*, segmental vessels.

Twelfth day.—Length 8·05 mm. (Fig. 12). Caudal fin (*c.f.*) is forked. Anal fin (*a.f.*) has two capillaries and appears as a ridge of the embryonic fin-fold just behind the anus. Pelvic fins are also marked off. Dorsal fin (*d.f.*) has six to seven rays with a set of capillaries.

Fourteenth day.—There are fifteen jointed and five or six unbranched caudal fin rays. Dorsal fin has 9 to 11, and anal 2 to 3 rays.

During the third week, the fry measured 9 to 10·5 mm. in length. On the 17th day caudal fin is seen separating off from the embryonic fold with the development of the anal fin (Fig. 17, *a.f.*, *c.f.*) and is fully separated off from the embryonic fold when the anal fin is completely developed, and is forked. It has 15 three-jointed rays and 5 to 6 short unbranched ones. Dorsal fin has 13 to 15 rays and is distinctly separated off from the embryonic fin-fold. Anal has 4 to 6 rays. Pelvic fins are underdeveloped. On the 21st day embryonic fin-fold is present ventrally but anal is separated from caudal. Round black pigment cells are present over the body and head.

Scales appeared on the 24th day after hatching and the fry possessed all the characters of the adult. A month old fry of *Cirrhina mrigala*, netted from a pond, measured 45 mm. with fully developed fins, while those reared in boxes or live cars measured 20 to 25 mm. in length during the same period.

Discussion

The development of *Cirrhina mrigala* illustrates some interesting features which require further consideration:—

(1) *Swelling up of egg.*—It is a remarkable phenomenon, and has previously been recorded by the author (1924, p. 959 and 1925, Fig. 7, Plate I) in the case of other cyprinidæ and figured in the case of *Labeo gonius*. It has also been described by Jones (1938, *b*) in *Garra ceylonensis ceylonensis*. As the egg swells a space is created between the outer membrane and the developing embryo. This space is filled with water and gives the egg a glassy bead-like appearance. The water cushion between the developing embryo and the egg membrane apparently protects the egg from external shock which may otherwise injure the embryo, especially when the eggs, which are laid loose, are likely to be carried down the streams by heavy floods.

(2) *Rapidity of embryonic development.*—Like most of the other Indian Cyprinoids, whose life-history is known (Hamid Khan, 1924, 1925 and Jones, 1938 *a* and *b*), hatching in *Cirrhina mrigala* takes 16 to 20 hours. The development, evidently, is quickened by the warmth of the sun falling

on the eggs lying in shallow water and, unlike the Western species, which hatch out in 12 to 16 days, the Indian carps take 16 to 20 hours to hatch. Newly hatched embryo in *Cirrhina mrigala* is underdeveloped and resembles that of *Labeo gonius* (Hamid Khan, 1925) and of *Danio malabaricus* (Jones, 1938, *a*), but differs considerably from that of *Garra* (Jones, 1938, *b*). It has neither a mouth, nor gills, nor gill clefts.

(3) *Development of caudal fin.*—Formation of caudal fin rays is preceded by the appearance of caudal capillaries lying ventral to the notochord in the embryonic fin-fold. On the second day after hatching, the caudal efferent vessel gives a branch which forms a loop and then flows back into caudal vein. Soon after, two loops are formed (Fig. 13, *c.c.*). Fourth day marks the appearance of fin rays between the capillary vessels (Fig. 14, *c.r.*). There are six to seven rays with four distinct and two indistinct basal cartilages on the fifth day (Fig. 15), and the number of rays increases daily with fresh formation of caudal capillaries. On the ninth day there are fifteen jointed and three to four unjointed caudal rays with six basal cartilages. As notochord curves dorsally the rays are drawn towards dorsal surface and heterocercal condition of the fin becomes apparent (Fig. 16, *c.f.*). The embryonic fin forms a dorsal lobe and does not contain any rays (Fig. 16, *e.f.*), while the ventral lobe contains fin rays and forms the permanent caudal fin. A similar condition has been described by Agassiz (1878) in the case of Flounders and other bony fishes. The tail fin thus seems to resemble the usual Elasmobranch form or still more that of some Ganoids, *e.g.*, the Sturgeon (Balfour, 1885). With the development of fresh fin rays the dorsal lobe of embryonic fin finally disappears. Caudal fin rays on the dorsal and ventral border of the fin grow rapidly than those in the middle of the fin and thus give to the fin its forked and externally homocercal appearance (Fig. 17, *c.f.*). On the 17th day, with the increased growth of anal fin, caudal fin is seen separating off from the embryonic fin-fold (Fig. 17, *a.f.* and *c.f.*).

(4) *Disparity in rate of growth.* Fry reared in confinement and those reared under natural condition in a pond show remarkable difference in their growth. On the 10th and 11th day the fry bred in confinement were still underdeveloped, with 4 to 5 unjointed caudal rays, while those reared in a shallow pond had fully developed dorsal fin with 15 rays, and well formed caudal, and pectoral fins. A month old fry reared in ponds under natural environments measures 45 mm. in length with fully developed fins, while those in confinement during the same period measured 20 to 25 mm. in length.

Rearing and feeding of fry is a delicate problem. If proper care is not taken, the fry are often attacked by external parasites. Feeding on artificial diet, such as wheat and gram flour, produced unhealthy conditions, while feeding on natural diet of rotifers and young crustaceens produced healthy fry.

Conclusion

The development of *Cirrhina mrigala* illustrates the helpless nature of eggs and fry and stresses the need to devise means to protect them from many dangers to which they are exposed. The eggs are laid during floods which may carry them to places where their fate becomes uncertain. The male sheds its milt in water and there are very great chances of some eggs being left unfertilized. The eggs lie submerged on the grass or sink to the bottom and there is every likelihood of the spawning fields drying up before they are hatched. If, however, the eggs escape all these misfortunes and hatch out in time and run into a pond or stream, there they are likely to fall an easy victim to their enemies, namely, the predaceous fish, frogs and birds. Establishment of nurseries and hatcheries near the spawning grounds could only ensure the development of eggs and fry and lessen the chances of their loss and destruction.

Summary

1. *Cirrhina mrigala* breeds in July when streams are flooded by monsoon rains. If the floods are untimely or insufficient to inundate the spawning fields, the fish do not spawn and become egg bound.

2. The egg measures 1·5 mm. but swells to 3 to 4 mm. when it falls in water. Embryonic development is rapid. The eggs hatch out from 16 to 19 hours. Heart appears just an hour or so before hatching. Newly hatched out embryo measures 3·8 to 4 mm. with a yolk sac, which is prolonged posteriorly. It possesses a pair of otocysts with two otoliths in each, auditory and optic vesicles, a pair of eyes without any pigment and one dorsal vessel, which turns back at its posterior extremity to form the caudal vein.

3. Pectoral fins appear on the first day. Mouth opens on the second day for respiration. Gut is formed on the third day and opens to the exterior. Yolk sac is completely absorbed on the fourth day.

4. Formation of the caudal rays is preceded by the appearance of caudal capillaries on the second day after hatching. The capillaries increase in number and it is on the fourth day that the rays appear. Dorsal fin rays appear on the seventh day, and anal and pelvic on the 12th day

after hatching. Scales appear on the 24th day when the fry have fully developed fins, and possess all the characters of the adult.

5. A month old fry reared in ponds measures 45 mm. in length, while those reared in confinement measure 20 to 25 mm. in length.

REFERENCES

Agassiz, A. .. "On the young stages of some Osseous Fishes. 1. Development of the tail," *Proc. Amer. Acad. Arts and Sci.*, 1877, **13**, 117–25.

Balfour, F. M. .. "A Treatise on Comparative Embryology," 1885, **2**, 79.

Hamid Khan .. "Observations on the breeding habits of some fresh-water fishes in the Punjab," *Bomb. Nat. Hist. Soc. Journ.*, 1924, **29**, 958-62.

———— .. "Early stages in the development of some fresh-water fishes in the Punjab," *Bomb. Nat. Hist. Soc. Journ.*, 1925, **30**, 532–40.

Jones, S. .. "On the breeding habits and development of a Cyprinid, *Danio* (*Danio*) *malabaricus* (Jerdon) in Ceylon," *Ceylon Journ. Sci.* (C), 1938, *a*, *b*, 79–89.

———— .. "External features in the embryonic development of a Ceylon Mountain Carp, *Garra ceylonensis ceylonensis* (Bleeker)," *Ceylon Journ. Sci.* (C), 1938, *b*, **6**, 91–98.

———— .. "Larval development of *Garra ceylonensis ceylonensis* (Bleeker)," *Rec. Ind. Mus. Cal.*, 1941, **43** II, 117–120.

THE ORIGIN AND DISTRIBUTION OF INTER- AND INTRAXYLARY PHLOEM IN *LEPTADENIA*

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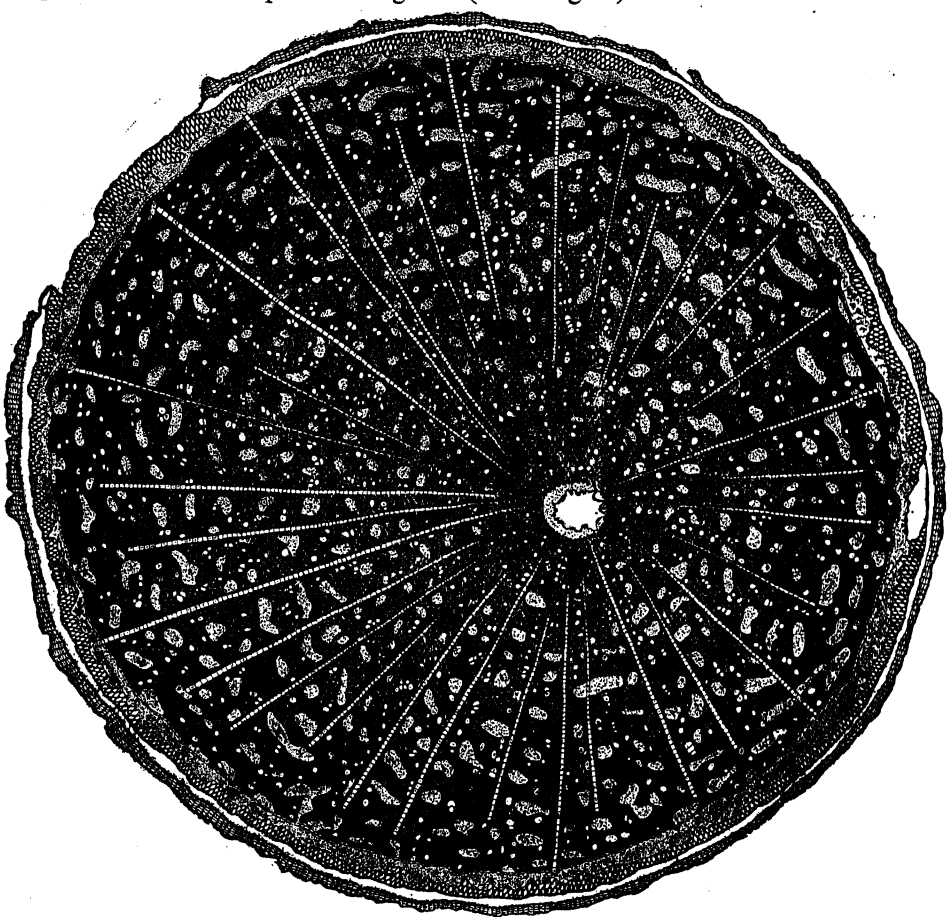
1. Introduction

ALTHOUGH intraxylary phloem is known to occur in a number of dicotyledonous families, the presence of interxylary phloem is comparatively rare. An examination of the stem of *Leptadenia spartium* Wight, collected from Agra, showed three phloem regions (Text-Fig. 1):—



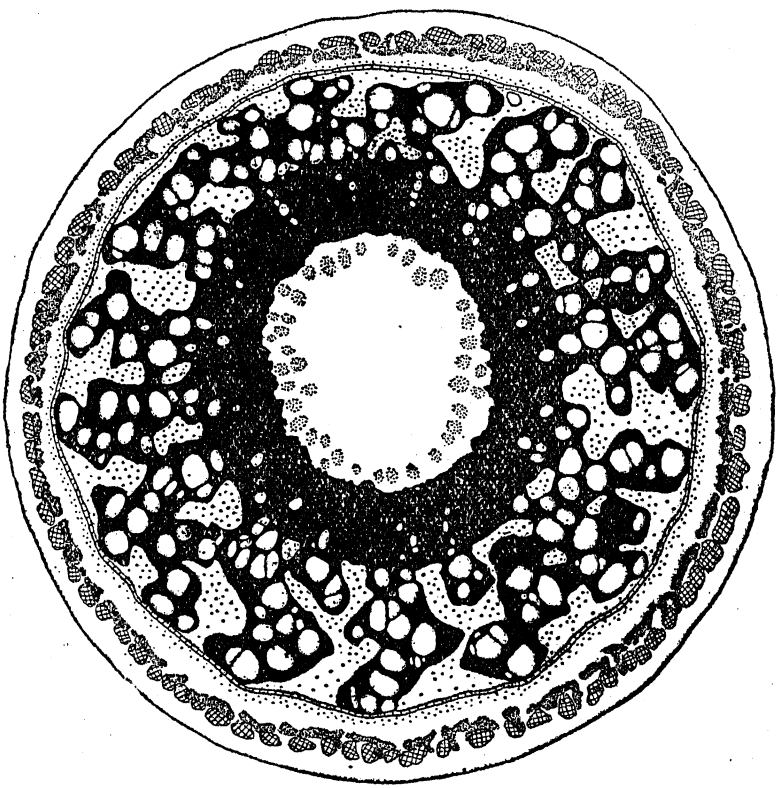
TEXT-FIG. 1. Outline drawing of transverse section of old stem of *L. spartium* ×6. In this and the next drawing the xylem parenchyma is represented by solid black, xylem vessels by empty spaces, cambium by a single line of cells, and the phloem islands by dotted portions within the wood.

(i) Outer normal phloem,

(ii) Intraxylary phloem adjacent to the pith, and

(iii) " Islands " of phloem scattered here and there in the secondary xylem.

Some material of *Leptadenia reticulata* W. and A., received from Prof. M. O. P. Iyengar of Madras, Prof. M. Sayeedud-Din of the Osmania University, and Mr. J. Venkateswaralu* of Coconada, was also examined and found to show the same abnormality (Text-Fig. 2), although other differences



TEXT-FIG. 2. Outline drawing of transverse section of stem of *L. reticulata* ×14.

were of course noticeable due to the shrubby habit of *L. spartium* and the climbing habit of *L. reticulata.* A reference to the available literature showed that no detailed account of the anatomy of *Leptadenia* is so far available. Of the previous workers only Maheshwari (1935) has called attention to the

* To all these gentlemen I offer my warmest thanks.

presence of the interxylary phloem; Sabnis (1921), Blatter, McCann, and Sabnis (1929), and Sayeedud-Din and Suxena (1940) make no mention of it. The only other genera of the Asclepiadaceæ in which interxylary phloem has been recorded are *Asclepias* L., *Morrenia* Lindl., and *Ceropegia* L. (Pfeiffer, 1926).

2. *Intraxylary Phloem*

In both the species the intraxylary phloem arises from the peripheral cells of the pith. New phloem cells continue to arise successively towards the outside even from the xylem parenchyma cells adjacent to the protoxylem elements, as in tobacco (Esau, 1938). The protoxylem elements are often crushed in the process and at places, in the thickest stems of *L. spartium* and *L. reticulata*, they could not be made out for that reason. In both the species there is also differentiated a cambial layer outside the internal phloem groups. This does not produce any secondary xylem, but the secondary phloem cut off by it towards the inside crushes some of the first formed phloem cells.

3. *Interxylary Phloem*

The phloem "islands" are not arranged in a regular order in either of the two species. They are smaller and fewer in the inner and older portions of the wood, but considerably larger and more numerous towards the periphery. In *L. spartium* (Plate I, Fig. 1) the islands are usually tangentially elongated but in *L. reticulata* (Plate I, Fig. 2) they have no definite shape. As many as 412 such islands were counted in a t.s. of the stem of *L. spartium* approximately 1·5 cm. in diameter. In *L. reticulata*, the number is appreciably smaller, for only 183 islands were seen in the cross-section of a stem 1·6 cm. thick.

A detailed study of the origin of the interxylary phloem revealed that at places the outer smooth boundary of the secondary xylem becomes indented (Plate I, Fig. 2) and the depressions are filled with bays of thin-walled tissue. This is because at these points the cambium begins to cut off, on the inside, groups of parenchymatous cells instead of secondary xylem. This is however only a temporary phase and it soon resumes its normal activity producing the usual secondary xylem elements (Plate I, Fig. 1). This process is repeated several times giving rise to a number of "islands" of thin-walled tissue embedded in the thick-walled cells of the wood. Some of the cells composing the islands, specially those in the centre of each island, are differentiated into sieve tubes and companion cells and the rest form phloem parenchyma.

As the islands become older and more deep-seated some of their central cells get crushed and degenerated. The obliterated tissue in *L. spartium* usually extends tangentially (Plate II, Fig. 3) or sometimes radially corresponding to the longer diameter of the island. In *L. reticulata* also the direction

of the crushed tissue varies with the shape of islands. Those cells of the medullary rays which happen to pass through the interxylary phloem groups, usually remain intact, however, even though the phloem cells on either side of them may be in an advanced state of degeneration. One reason for this peculiar crushing of the central phloem cells is that, in the outer parenchyma cells of the older islands, divisions accompanied by subsequent differentiation and enlargement of additional sieve tubes and companion cells continue to take place for a fairly long time. As the islands are surrounded by thick-walled xylem parenchyma, the expanding phloem elements cannot spread outwards and the whole force is therefore directed centripetally resulting in a crushing of the central cells.

A weak cambium-like layer† has occasionally been observed around some of the old phloem islands in the stems of *L. spartium* (Plate II, Fig. 3) as well as *L. reticulata* (Plate II, Fig. 4). It may begin to differentiate on any side or more than one side of the island. If it develops first on the inner side, the islands may partially resemble those of *Strychnos* although they originate quite differently in this case. As found by Hérail (1885) and subsequently confirmed by Scott and Brebner (1889), the phloem islands are here produced centrifugally by portions of the normal cambium which afterwards become slackened in activity. At the outer borders of these groups, however, new cambial segments arise from the pericycle or phloem parenchyma. These " complementary segments " bridge over the groups and join on to the main cambium, thus completing the general ring. Like the rest of the cambium they now cut off secondary xylem towards the inner and secondary phloem towards the outer sides. As a result, the groups of phloem lying below those segments get embedded in the xylem. The two processes continue to alternate so that an old stem shows quite a large number of phloem islands, each having a centripetally embedded cambium.

4. *Comparison with the Phloem Islands of* Strychnos

It is worthy of note that in a young stem of *Leptadenia* the phloem cells constituting the islands lie in the same radial rows as the cells of the wood both centripetally as well as centrifugally (Plate I, Figs. 1 & 2). In *Strychnos nux-vomica*, on the other hand, Scott and Brebner (1889) found that while the radial rows could readily be traced from the interxylary phloem into the wood on the inner side, no such continuity existed in the opposite direction. This difference is clearly due to the different modes of origin of the interxylary

† A secondary cambium has also been reported around the groups of phloem cells in the roots of certain Cruciferæ (Weiss, 1883); *Salvadora*, and some Cucurbitaceæ (Scott and Brebner, 1889); and *Ipomœa batatas* (Artschwager, 1924).

phloem in the two genera. In *Leptadenia* the islands as well as the xylem cells on its outer and inner sides are produced from the same cambium. In *Strychnos*, on the other hand, the island and the inner wood are products of the same cambium, but the cells of the outer wood are formed from the new complementary cambial segments, and do not therefore lie in the same radial rows as the cells of the interxylary phloem towards the inside.

Another difference is seen in the islands themselves. In *Strychnos*, in the older islands, the crushed phloem forms a sort of a cap on its outer side due to the activity of the embedded cambial segment which was once a part of the original cambium ring. This continues to add some new cells towards the outside causing pressure upon the older cells. In *Leptadenia*, on the contrary, the crushed phloem occupies the central place in the island, for reasons which have already been explained above.

5. Summary

1. In *Leptadenia spartium* and *L. reticulata* there are three phloem regions: (*a*) the outer normal phloem, (*b*) the intraxylary or inner phloem, and (*c*) the interxylary phloem which forms inclusions in the wood.

2. The patches of intraxylary phloem arise from the pith cells, but in later stages even the xylem parenchyma cells adjacent to the pith take part in their formation. In old stems a cambium is differentiated on the outer faces of these phloem groups and produces some secondary phloem centripetally.

3. The interxylary phloem, present in the stem, becomes differentiated from groups of thin-walled cells produced centripetally by the cambium. Later the cambium resumes its normal activity with the result that the phloem groups become embedded in the secondary xylem.

4. Owing to an enlargement of the cells in the island and the fact that it is surrounded on all sides by the woody cells of the xylem, there is often a compression and crushing of the phloem tissues in its centre.

5. A weak secondary cambium has occasionally been observed to differentiate on one or more than one side of some of the older phloem islands.

6. A comparison with *Strychnos nux-vomica* shows that in the latter the islands are always produced centrifugally from the cambium and later become embedded due to the formation of a complementary cambial segment on the outer side, whereas in *Leptadenia* it is the same cambium which produces both the secondary xylem as well as the phloem on its inner side.

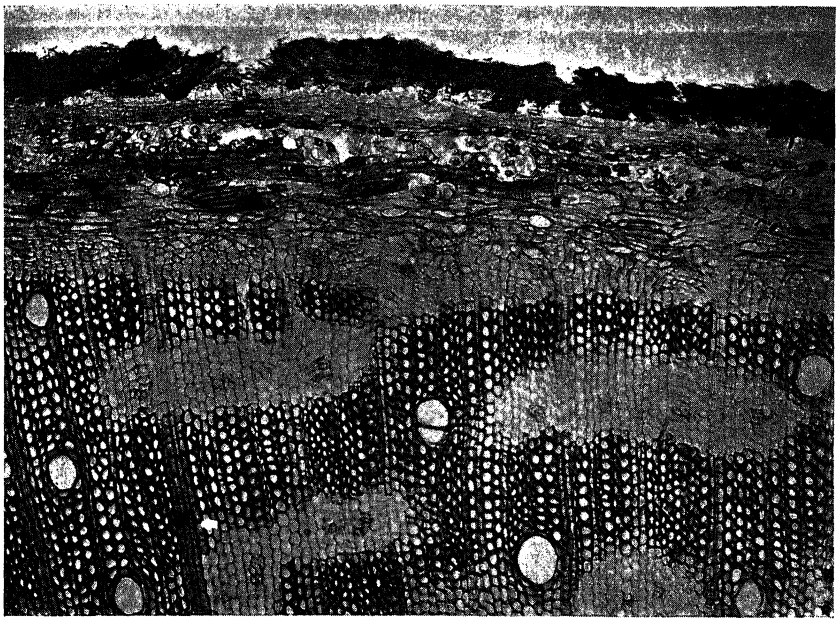


FIG. 1

L. spartium—outer portion of an old stem showing some newly formed islands of phloem. Note their tangential elongation. ×65.

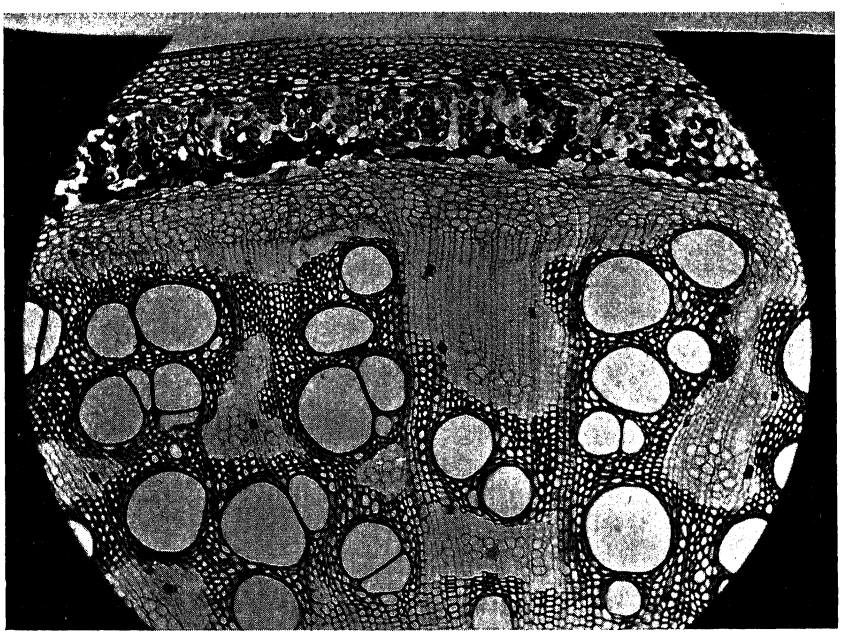


FIG. 2

Same in *L. reticulata*. Note the irregular shape of the islands. ×47.

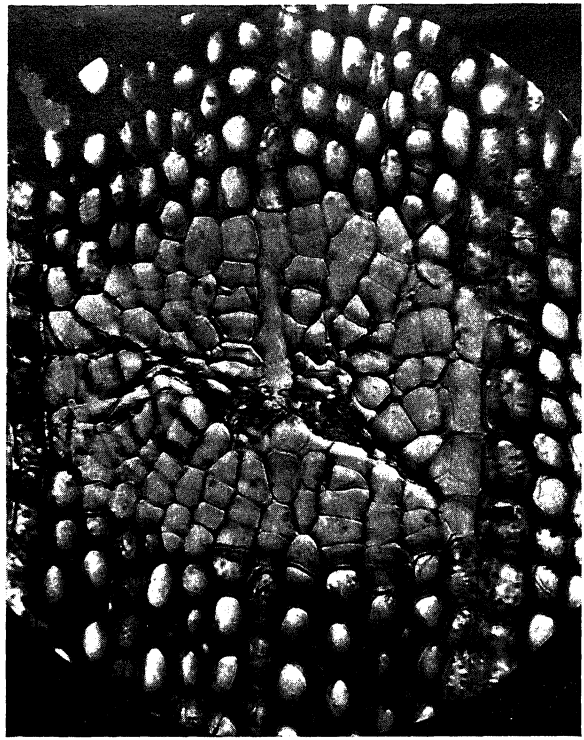


FIG. 3

L. spartium—An advanced stage showing the tangential crushing of the central cells of the island. ×290.

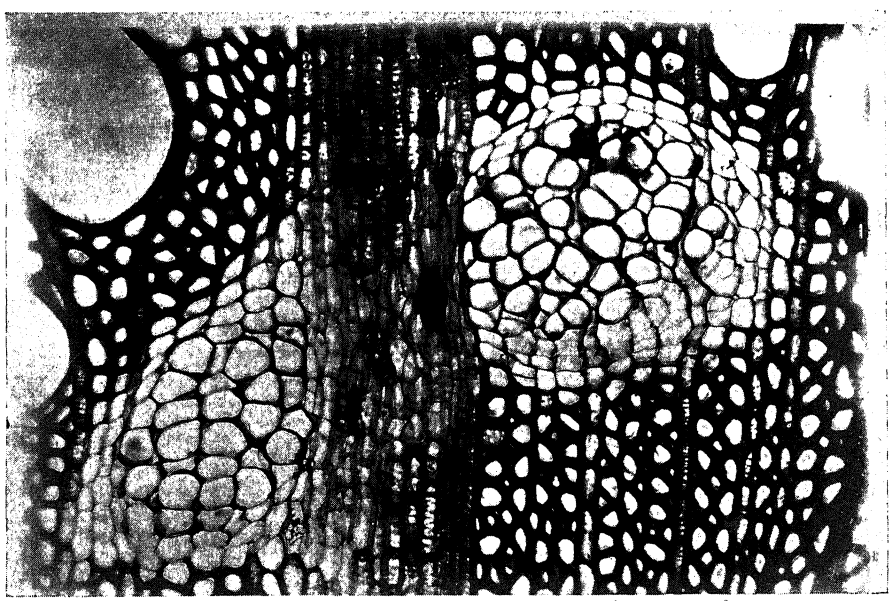


FIG. 4

Phloem islands in *L. reticulata* ; note the cambium-like cells on the inner as well as outer sides of the island on the right. ×175.

6. Acknowledgements

I express my heartfelt thanks to my teacher, Dr. P. Maheshwari, for the guidance and help which he has so kindly rendered from time to time. I am also indebted to my friend, Mr. Sukumar Sen, for helping me in taking the micro-photographs, and to Prof. B. Sahni for his kindness in going through the manuscript.

LITERATURE CITED

1. Artschwager, E. .. "On the anatomy of the sweet potato root with notes on internal breakdown," *Jour. Agri. Res.*, 1924, **27**, 157–166.
2. Blatter, E., McCann, C., and Sabnis, T. S. "The flora of the Indus Delta," *Jour. Ind. Bot. Soc.*, 1929, **8**, 19–61.
3. De Bary, A. .. "Comparative Anatomy of the Vegetative Organs of the Phanerogams and Ferns," Engl. Trans., Oxford, 1884.
4. Esau, K. .. "Ontogeny and structure of the phloem of tobacco," *Hilgardia*, 1938, **11**, 343–424.
5. Hérail, J. .. "Recherches sur l'anatomie comparée de la tige des Dicotylédones," *Ann. Sci. Nat. Bot.*, 1885, **7**, 2nd ser., 203–314 (Quoted from Scott, D. H., and Brebner, G., 1889).
6. Maheshwari, P. .. "The presence of interxylary phloem in the stem of *Leptadenia spartium* Wight," *Proc. Ind. Sci. Cong.*, 22nd Session, 1935, Calcutta.
7. Pfeiffer, H. .. "Das abnorme Dickenwachstum." In Linsbauer's *Handbuch der Pflanzenanatomie*, Borntträger, Berlin, 1926.
8. Sabnis, T. S. .. "The physiological anatomy of the plants of the Indian desert," *Jour. Ind. Bot. Soc.*, 1921, **2**, 1–19.
9. Sayeedud-Din, M., and Suxena, M. R. "On the anatomy of some of the Asclepiadaceæ," *Proc. Nat. Acad. Sci.*, India, 1940, **10**, 129–132.
10. Scott, D. H., and Brebner, G. "On the anatomy and histogeny of *Strychnos*," *Ann. Bot.*, 1889, **3**, 275–304.
11. Solereder, H. .. "Systematic anatomy of the Dictoyledons," Engl. Transl. Oxford, 1908.
12. Weiss, J. E. .. "Das markständige Gefässbündelsystem einiger Dikotyledonen in seiner Beziehung zu den Blattspuren," *Bot. Cbl.*, 1883, **15**, 280–295, 318–327, 358–367, 390–397, 401–415.

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STUDIES ON MYXOSPORIDIA FROM THE COMMON FOOD FISHES OF BENGAL

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CONTENTS

Introduction

THE author has been studying, for the last few years the myxosporidian parasites of fishes, amphibians and reptilians obtainable in the vicinity of Calcutta and some of his previous observations were recorded before (1939, 1941). The present paper deals with a number of myxosporidians parasitic in the common food fishes of Bengal. The majority of the parasites described here are new, while a few call forth fresh attention. *Myxidium leiberkühni* Bütschli (1881) for instance, has been reported from various fishes inhabiting different parts of the world except India. Now it is reported for the first

time from India too but from a new host *Anabas testudineus*. Another species *Chloromyxum amphipnoui* though reported by Ray* (1933) from India but there is no account of it. A description of the latter, therefore, is also added here.

The fishes harbouring the parasites were either collected from the local tanks or purchased from Calcutta markets. Some of the fishes were kept in large aquaria in the laboratory.

The parasites, specially the spores, were observed in fresh conditions following the improved method given by Nemeczek (1926) and their measurements were taken. For extrusion of filaments, 1 to 10 per cent. KOH solution or Methyl Alcohol-giemsa method of the author (1939) proved satisfactory. Lugol's solution was used for detecting the iodinophilous vacuole and the nuclei. Smears of infected organs and tissues were fixed in Schaudinn's fluid as well as in Bouin-Duboscq. The latter was, however, mainly used for fixation of tissues meant for cutting sections. Delafield's and Iron-alum hæmatoxylin were used for staining both the smears and sections.

The number of fishes examined, the number of fishes parasitised, as well as a list of the parasites with their seat of infection and locality are given in Table I.

Description of Species

Leptotheca latesi n.sp.

(Figs. 1–7)

Host: *Lates calcarifer* (Bloch.). Of the five fishes examined, three were found infected.

Seat of Infection: Gall-bladder.

Locality: Bengal.†

Vegetative Form.—Both mature and young trophozoites are found in the permanent preparations of the gall-bladder of the host. The mature trophozoites (Figs. 1, 2) are circular in outline measuring 10–14 μ in diameter.

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* I am indebted to Dr. H. N. Ray, Systematic Protozoologist, Imperial Institute of Veterinary Research, Mukteswar, for kindly placing his materials at my disposal for further investigation. Thanks are also due to Mr. D. Mukherji and Mr. G. K. Chakravarti who helped me in various ways.

† As it is difficult to ascertain the exact locality of the fishes purchased from markets owing to their being imported from different parts of the Province, 'Bengal' has been used as their locality.

No distinction could be made between the ectoplasm and the endoplasm of the parasites, the cytoplasm being uniformly granular. The youngest forms (Fig. 3) are also more or less circular in outline and contained two nuclei. Developing trophozoites (Fig. 4) with two to eight nuclei could also be observed in the smear preparation. Fully formed trophozoites contained two mature spores (Fig. 2), they are therefore disporous.

THE SPORE.—The mature spores (Figs. 5–7) are bean-shaped in lateral view with both the extremities rounded, while the developing young spores are more or less crescent-shaped. The valves of the spore are smooth and symmetrical. The sutural line is prominent both in fresh and stained specimens, but the sutural ridge could not be seen. The polar capsules are spherical in shape and equal in size; they are located one on each side of the sutural line and are surrounded by a delicate membrane. The sporoplasm which is granular, does not occupy the entire extracapsular cavity of the spore and is situated below the polar capsules. It has the form of a triangle, the apex of which is directed downwards and contains the two nuclei distinctly visible in stained preparations (Fig. 7). Dimensions: breadth of the spore $10 \cdot 3 - 12 \cdot 4\,\mu$, sutural diameter of the spore $6 \cdot 2\,\mu$, polar capsules $3 \cdot 1\,\mu$ in diameter, polar filament 50–80 μ long.

REMARKS.—The myxosporidian under report does not resemble any known species of *Leptotheca.* Its spores approach those of *L. inconstans* (Jameson, 1929), *L. agilis* Thélohan (1895) in size and to those of *L. informis* Auerbach (1910) and *L. longipes* Auerbach (1910) in shape.

Leptotheca macronesi n.sp.

(Figs. 8–11)

HOST: *Macrones gulio* (Ham.). Of five fishes examined, two were found infected.

SEAT OF INFECTION: Gall-bladder.

LOCALITY: Bengal.

VEGETATIVE FORM.—Only a few mature trophozoites (Fig. 8) were found in living condition. They are circular in outline with an uniformly granular cytoplasm, containing several refractile granules. The trophozoites are monosporous and measure $10 \cdot 3\,\mu$ in diameter.

THE SPORE.—The spores (Figs. 9–11) are elliptical in shape with the ventral side more or less flattened; their extremities are rounded but one of them is slightly narrower than the other. The valves are smooth, unequal and thin. The sutural line is fine. The equal and spherical polar capsules

are placed one on either side of the sutural line, and are surrounded by a delicate membrane. The granular sporoplasm occupies the entire extracapsular cavity of the spore and contains two nuclei. Capsulogenous nuclei are present at the bases of the capsules. Dimensions: breadth of the spore 10–14·4 μ, sutural diameter 6·18–7·2 μ, polar capsules 3·1 μ in diameter.

REMARKS.—The breadth of the majority of the spores is twice the sutural diameter, but in a few it is less than twice while in others it is slightly larger than sutural diameter. From the definition of the genera *Leptotheca* and *Ceratomyxa*, it seems that the myxosporidian under report stands in an intermediate position between the two genera.

L. macronesi n.sp. shows affinities to *L. constricta* Fujita (1923), *L. fisheri* (Jameson, 1929), and *L. inconstans* (Jameson, 1929).

Ceratomyxa scatophagi n.sp.

(Figs. 12–17)

HOST: *Scatophagus argus* (Bloch.). Among twenty-five fishes examined, ten were found infected.

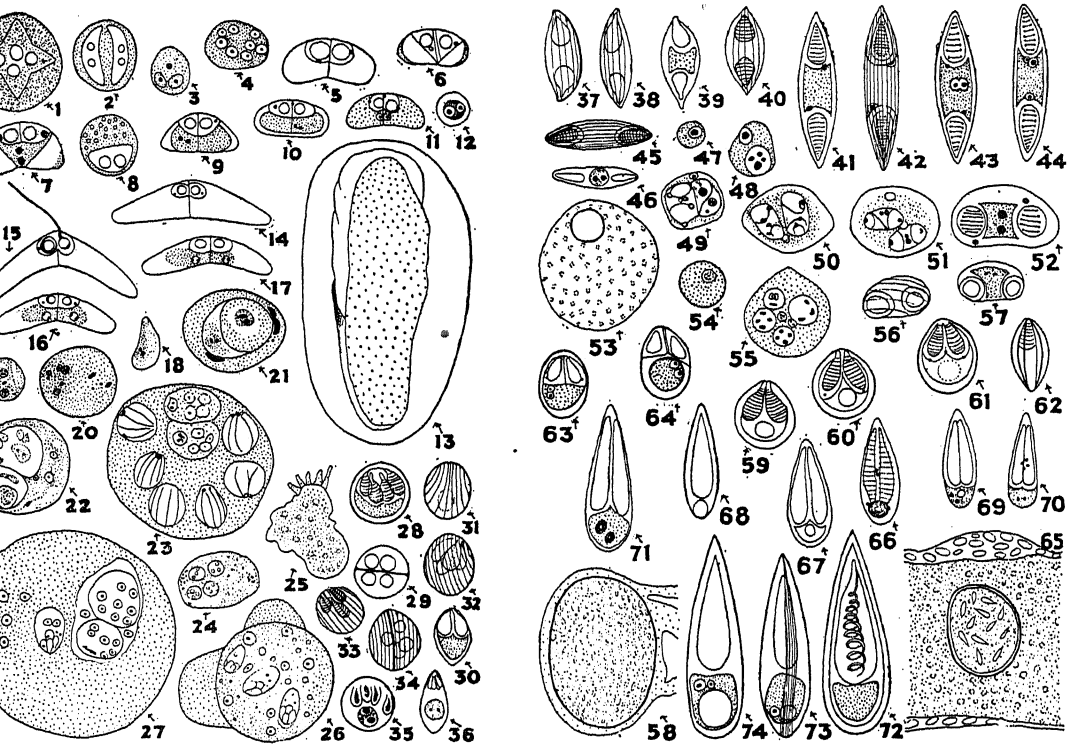
SEAT OF INFECTION: Gall-bladder.

LOCALITY: Bengal.

VEGETATIVE FORM.—The youngest forms (Fig. 12) observed in the smear preparations are circular in outline and are binucleate; the central granular endoplasm is surrounded by the hyaline ectoplasm. They measure 5 μ in diameter. Mature forms measuring 50–120 μ × 40–85 μ, are irregular in shape and the body can be distinguished into an outer hyaline ectoplasm and an inner granular endoplasm having nuclei irregularly scattered in it (Fig. 13). Unfortunately the sporulating trophozoites could not be found.

THE SPORE.—The spores (Figs. 14–17) are crescent-shaped in lateral view. The valves are alike being cylindrical in form with the terminal extremities rounded. The curvature of the valves varies to some extent from almost straight to concave. The sutural line is faintly marked. The polar capsules are equal and spherical and extrude the filament when treated with 10 per cent. KOH solution. The sporoplasm does not fill the entire extracapsular cavity of the spore, but is situated asymmetrically beneath the polar capsules and contains two nuclei (Figs. 16, 17). Dimensions: breadth of the spore 16–26 μ, sutural diameter of the spore 4·2–7·2 μ, polar capsules 2·5–3·1 μ in diameter, polar filament 30–50 μ in length.

REMARKS.—Awerinzew (1913) reported an unnamed *Ceratomyxa* from the gall-bladder of *Scatophagus argus* collected from Delagoa Bay, Africa. The



Figures were drawn under a camera lucida and magnified 833 times, unless otherwise stated.

Figs. 1–7. *Leptotheca latesi* n.sp.—Fig. 1. A fresh trophozoite. Fig. 2. A stained trophozoite containing spores. Fig. 3. A young trophozoite from a stained smear. Fig. 4. A stained developing trophozoite. . 5–6. Lateral view of fresh spores. Fig. 7. A stained spore showing sporoplasm and nuclei. × 1350. . 8–11. *Leptotheca macronesi* n.sp. Fig. 8. A fresh trophozoite. Figs. 9–11. Lateral view of fresh spores. . 12–17. *Ceratomyxa scatophagi* n.sp. Fig. 12. Youngest trophozoite from a stained smear. Fig. 13. A ıed mature trophozoite. Figs. 14–15. Fresh spores, fig. 15 shows an extruded filament. Figs. 16–17. Stained es. Figs. 18–36. *Chloromyxum amphipnoui* Ray.—Figs. 18–24. Trophozoites from *A. cuchia*; figs. 18–19, ıed youngest trophozoites; figs. 20–23, developing trophozoites from stained smear; fig. 24, a fresh tropho- e. ×365. Figs. 25–27. Trophozoites from *H. fossilis*; fig. 24, a fresh trophozoite ×365; figs. 26–27, ıed trophozoites. Figs. 28–34. Fresh spores; figs. 28 and 33, front view; fig. 30, side view; fig. 29, top showing sutural line; figs. 31–34, showing striations. Figs. 35–36. Stained spores. Figs. 37–40. *Myxidium ro pneustesi* n.sp.—Figs. 37–39. Front view of fresh spores. Fig. 40. Side view of a fresh spore. Figs. 44.—*Myxidium procerum* var. *calcarifera* var. n. Figs. 41–44. Fresh spores; fig. 42. showing striations; note position of nuclei in figs. 43 and 44. Figs. 45–46. *Myxidium leiberkūhni* Bütschli.—Fig. 45. A fresh spore. 46. A stained spore. Figs. 47–52. *Zschokkella fossilæ* n.sp.—Fig. 47. A stained youngest trophozoite. . 48–49. Stained trophozoites showing developing pansporoblast. Figs. 50–51. Stained mature trophozoites. 52. A fresh spore. ×1750. Figs. 53–57. *Zschokkella ilishæ* n.sp.—Fig. 53. A fresh trophozoite. Fig. 54. tained youngest form. Fig. 55. A developing trophozoite from a stained smear. Figs. 56–57. Fresh es. Figs. 58–64. *Myxobolus clarii* n.sp.—Fig. 58. A cyst from a section of testis. ×55. Figs. 59–61. nt view of fresh spores. Fig. 62. Side view of a fresh spores. Figs. 63–64. Stained spores. Figs. 65–71. *xobolus catlæ* n.sp.—Fig. 65. A cyst within the gill filament. From a section. ×360. Fig. 66. Front view fresh spore. Fig. 67. Iodine stained spore showing iodinophilous vacuole. Fig. 68. Side view of a fresh e. Figs. 69–70. Stained spores. Fig. 71. Stained spore. ×1350. Figs. 72–74. *Thelohanellus rohitæ* (Southwell Prasad).—Fig. 72. Front view of a fresh spore. Figs. 73–74. Stained spores; sutural ridge is shown ig. 73 (side view).

description and size of the vegetative forms given by him do not agree with the myxosporidian under consideration. Further, owing to the want of any description of the spores of the African form it is difficult for me to call these two myxosporidians synonymous. The spores of the parasite under report approach those of *C. menospora* Davis (1917) in size but they differ in shape. Moreover, the vegetative forms of these two species differ to a considerable extent. The spores of the present *Ceratomyxa* have some affinities with that of *C. urophysis* Fantham, Porter and Richardson (1940).

*Chloromyxum amphipnoui** Ray

(Figs. 18–36)

HOST.—*Amphipnous cuchia* (Ham. Buch.) and *Heteropneustes fossilis* (Bloch.). The hosts were kept alive for observation under laboratory conditions. Eight eels were examined and of these five were infected. Of the twelve cat fishes examined five were infected with *C. amphipnoui*, and two other hosts carried both *C. amphipnoui* as well as another species of *Myxidium* which is described elsewhere in this paper.

SEAT OF INFECTION: Gall-bladder.

LOCALITY: Calcutta.

VEGETATIVE FORM.—The trophozoites in the two hosts differ slightly in their structure, they are therefore described separately below.

In the eel *A. cuchia* the youngest forms (Figs. 18, 19) obtained from the permanent preparations of the gall-bladder are amœboid in shape. They are binucleate and in maximum width measure 4·12–8·24 μ. As these forms increase in size, they become oval or spherical in shape with nuclei dividing. In forms measuring 12·36 μ in diameter, 8–10 nuclei are generally found irregularly scattered in the cytoplasm which appears uniformly granular (Fig. 20). The nuclei later arrange themselves into groups and give rise to the pansporoblast (Figs. 21, 22).

The pansporoblast gives rise to two sporoblasts. The nuclei within the sporoblast divide and give rise to a single spore. The mature trophozoites vary from 14·4 $\mu \times$ 16·5 μ – 35 $\mu \times$ 40 μ in size and the smaller ones contain two mature spores while the bigger ones eight (Fig. 23).

Trophozoites of various stages and sizes containing developing and mature spores have been examined in living condition. They are oval or spherical in outline (Fig. 24) and do not produce any pseudopodia and

* The specific name as given by Bhatia (1938) is '*amphipnovi*' and I have retained for obvious reasons the name as given by the original author.

seemed non-motile. The cytoplasm of these parasites appear uniformly granula without any marked distinction between the ectoplasm and the endoplasm. There are, however, several refractile bodies scattered irregularly in it.

In the fresh smear preparations of the gall-bladder of *H. fossilis*, amœboid trophozoites were noted in the living condition and they exhibited a very sluggish streaming movement (Fig. 25). During the movement the parasites give out digitiform pseudopodia and curiously enough these are generally formed at the end opposite to the direction of movement. Though in locomotion they differ from the parasites of *A. cuchia* to which they closely resemble in the structure of the cytoplasm.

In permanent preparations only, fully formed trophozoites (Figs. 26, 27) are found in large numbers. Pansporoblasts are formed within the trophozoites and the nucleus of the former divides into two, four, eight and sixteen. The pansporoblast then divides into two sporoblasts each of which contains eight nuclei (Fig. 27). As in the other host a single spore is developed in each sporoblast.

In both the hosts therefore the pansporoblasts are disporoblastic while the sporoblast is monosporic.

THE SPORE.—The shape and size of the spores are exactly alike in both the hosts. In shape they are almost spherical in front view (Figs. 28, 33), and ovoidal in side view (Fig. 30), but when seen from the top at certain angles they appear rectangular. The valves of the shell are alike and are sculptured by striations (Figs. 31–34). These striations give various pictures when seen from different angles as shown in the figures. In top view they run parallel to the sutural line. The sutural line is very fine and is only visible when seen from the top (Fig. 29). The polar capsules are of equal size, oval in shape but anterior end attenuated. They are placed side by side within the spore, their attennuated end directed toward the anterior end of the spore. The capsules are provided with distinct coiled filament inside; the latter is visible in a fresh spore. The sporoplasm occupies the entire extracapsular space of the spore and when stained it shows two distinct spherical nuclei (Figs. 35, 36). Dimensions: length or breadth of the spore 8·24–10·3 μ, polar capsules 4·1–5·2 $\mu \times$ 3·1–4 μ, polar filament 35–50 μ long.

REMARKS.—The parasite described above was reported by Ray (1933) from the gall-bladder of *Amphipnous cuchia*. The same myxophoridian was also observed by me in the gall-bladder of the same host as well as in the gall-bladder of *Heteropneustes fossilis*. Although the vegetative forms from the two hosts differ in their structure in some respects, yet the spores of the parasites found in these two hosts are exactly alike both in shape and size.

C. amphipnoui shows close affinities with *C. chitosense* Fujita (1923) both in form and size of the vegetative forms as well as in the structure of the spores but the former differs in having striations on the valves of the spores, all the polar capsules being equal in size, and having spherical nuclei in the sporoplasm. It also resembles in some character *C. fluviatile* Thélohan (1892), *C. dubium* Auerbach (1908), *C. trijugum* Kudo (1920), *C. catostomi* Kudo (1920) and *C. sphæricum* Fujita (1927).

Myxidium heteropneustesi n.sp.

(Figs. 37–40)

HOST: *Heteropneustes fossilis* (Bloch.). Only two fishes were found infected.

SEAT OF INFECTION: Gall-bladder.

LOCALITY: Calcutta.

VEGETATIVE FORM.—Not found.

THE SPORE.—The spores (Figs. 37–40) are more or less spindle-shaped with bluntly pointed extremities. The valves are provided with fine striations, and the sutural line cannot be distinguished from them. The polar capsules are slightly ovoidal with their anterior ends pointed. They are equal in size and are provided with coiled filaments which are clearly seen in fresh spores. The granular sporoplasm in live spores is rectangular in shape. It is situated in the space between the polar capsules. Dimensions: length of the spore 14·42 μ, breadth of spore 6·18 μ, polar capsules 4·12–6·18 μ $\times$ 4·12 μ.

REMARKS.—The shape of the spores of the parasite under report does not resemble any known species of *Myxidium* so far described, but in size the spores approach to forms such as *M. oncorhynchi* Fujita (1923), and *M. kudoi* Meglitsch (1937). The spores show affinities to those of *M. giardi* Cépède, given by Schäferna and Jirovec (1934).

Myxidium procerum var. *calcariferi* var. n.

(Pl. II, Figs. 41–44)

HOST: *Lates calcarifer* (Bloch.). Five fishes were examined, only one was infected with this myxosporidian, and the three other with *L. latesi.*

SEAT OF INFECTION: Gall-bladder.

LOCALITY: Bengal.

VEGETATIVE FORM.—Not found.

THE SPORE.—The shape of the spores is elongated and fusiform with terminal ends pointed (Figs. 41–44). The valves are equal and marked

with longitudinal striations, which run parallel to one another in the middle region of the spore, but converge at the pointed ends (Fig. 42). The sutural line could not be seen either in fresh or stained spores. The polar capsules are typically pyriform in shape and equal in size. The polar filaments are well marked in fresh condition and are extruded when treated with Methyl Alcohol-giemsa method. The sporoplasm occupies the entire space of the spore between the polar capsules, and contains two nuclei lying either side by side at the centre of the sporoplasm (Fig. 43), or placed at the base of the polar capsules widely separated from each other (Fig. 44). Capsulogenous nuclei at the base of the capsules are also distinctly marked in living condition. Dimensions: length of the spore 23–27 μ, breadth of the spore 6·18 μ, polar capsules 8·24 μ × 4·12 μ, polar filament 25 to 30 μ in length.

REMARKS.—The shape and size of the spores of the myxosporidian under report are exactly like those of *Myxidium procerum* Auerbach (1910 *a*). The species under consideration, however, differs from the latter in having striations on the valves and also with regard to the position of the sporoplasm. Moreover it is found in a different host. As the structural difference is a minor one I propose to call it a new variety of *M. procerum.*

Myxidium leiberkühni Bütschli (1881)

(Figs. 45–46)

HOST: *Anabas testudineus* (Bloch.). Of the ten fishes examined only two were found infected with this parasite.

SEAT OF INFECTION: Gall-bladder.

LOCALITY: Calcutta.

VEGETATIVE FORM.—Not found.

THE SPORE.—The spores (Figs. 45–46) are fusiform in shape with both the extremities pointed as described by previous workers. The valves of the spore are thin and marked with longitudinal striations. The shell though delicate is highly resistant, since 10 per cent. KOH solution could not penetrate the spore to bring about the extrusion of the polar filaments. The sutural line is very faint and can be seen with certain difficulty. The polar capsules are pyriform in shape and equal in size. The sporoplasm contains two nuclei and occupies the space in between the polar capsules. The size of the spores obtained by me approach to that given by Fujita (1924). Dimensions: length of the spore 12·4–15 μ, breadth of the spore 4·12–5 μ, polar capsules 4·12 μ × 2·06 μ, polar filaments 15 μ in length.

Zschokkella fossilæ n.sp.

(Figs. 47–52)

HOST: *Heteropneustes fossilis* (Bloch.). Twelve fishes were examined but only one was found infected.

SEAT OF INFECTION: Gall-bladder.

LOCALITY: Calcutta.

VEGETATIVE FORM.—The trophozoites are roughly circular in outline and in fresh conditions they do not exhibit any movement. The ectoplasm and the endoplasm are distinguishable both in live and stained specimens; the former appears non-granular while the latter is highly granular and contains nuclei of the different stages, as well as the developing spores. The youngest trophozoite (Fig. 47) when examined in smear preparation is 4·12 μ in diameter and is spherical in shape, having the nucleus placed excentrically and cytoplasm granular. The nucleus has a definite nuclear membrane with a spherical karyosome. As the trophozoite grows, it gives rise to two pansporoblasts, each of which is transformed into a sporoblast originating in a single spore (Figs. 48–49). The fully matured trophozoite (Figs. 50–51) contains two spores and measures 12·36–16·48 μ in diameter.

THE SPORE.—The spores (Fig. 52) are more or less semi-circular in outline with one of the sides more or less straight or slightly concave and the angles rounded. The valves of the shell are thin and are not provided with any striations. The sutural line is in the form of an elongated 'S'. The polar capsules are placed at each end of the spore; they are spherical and equal in size having distinct coiled filaments. The sporoplasm is more or less rectangular in shape, granular in texture and contains two distinct nuclei. Dimensions: length of the spore 10·3 μ, breadth of the spore: 4·12–5·18 μ, polar capsules 3·1 μ in diameter.

REMARKS.—*Zschokkella fossilæ* n.sp. shows close affinities to *Z. globulosa* Davis (1917), but differs from the latter in the following points. Trophozoites of *Z. fossilæ* have a distinct ectoplasm, are non-motile and do not produce any pseudopodia, while those of *Z. globulosa* have cytoplasm homogeneous and are slowly amœboid giving out short lobose pseudopodia. Moreover there is a difference between their spores.

Zschokkella ilishæ n.sp.

(Figs. 53–57)

HOST: *Hilsa ilisa* (Ham.). Six fishes were examined and of these only one was found infected.

SEAT OF INFECTION: Gall-bladder.

LOCALITY: Bengal.

VEGETATIVE FORM.—The live trophozoites (Fig. 53) are disc-shaped, non-motile and have uniformly granular cytoplasm. The stained specimens contain developing pansporoblasts (Fig. 55). The trophozoites in longest diameter measure 14·5–22·6 μ. The youngest forms obtained are uninucleate, and are circular in outline; they measure 4·12–6·18 μ in diameter (Fig. 54). Other stages could not be found.

THE SPORE.—The spores (Figs. 56, 57) are more or less semi-circular in shape, the basal line being slightly concave with the ends rounded. The valves are thin and are provided with longitudinal striations. The polar capsules are equal and spherical. The sporoplasm is situated in between the polar capsules but dorsally extends over the capsules. It contains two small nuclei. Dimensions: length of the spore 12·36 μ, breadth of the spore 6·18 μ, polar capsules 4·26 μ in diameter.

REMARKS.—The spores of *Z. ilishæ* n.sp. closely resemble in shape and size those of *Z. fossilæ* described above, but differ in having striations on the valves and in the structure of the sporoplasm. Further the vegetative forms of these two species are different to some extent.

Myxobolus clarii n.sp.

(Figs. 58–64)

HOST: *Clarius batrachus* (Linn.). Of twelve fishes examined, nine were found infected.

SEAT OF INFECTION: Gall-bladder, liver, testes, ovary and fat-bodies. All the infected fishes in their gall-bladder harboured mature spores while cysts were found distributed in the liver, fat-bodies and gonads.

LOCALITY: Calcutta.

VEGETATIVE FORM.—The only forms that could be obtained were the cysts and almost all of these contained mature spores. The cysts (Fig. 58) are broadly oval in shape and appear opaque white when seen under the microscope in living condition. They are surrounded by a cyst-membrane about 4–6 μ in thickness and they measure 780–975 μ × 604–877 μ.

THE SPORE.—The shape of the spores are subspherical in front view (Figs. 59–61) and lenticular in lateral view (Fig. 62). The shell of the spore is comparatively thick with equal valves. The sutural ridge is distinctly marked in fresh spores. The polar capsules are pyriform with their anterior

ends drawn out into a short narrow tube and exhibit distinctly the coiled filament. The sporoplasm which appears granular in fresh spores, is situated behind the polar capsules and occupies the entire extracapsular cavity of the spore. A spherical iodinophilous vacuole is present within it. Two nuclei are found in the sporoplasm of stained spores (Figs. 63, 64). Capsulogenous nuclei are also seen attached to the bases of the capsules. Dimensions: length of the spore 11·3–12·4 μ, breadth of the spore 10·3 μ, thickness of the spore 6·18 μ, sutural ridge 2·06 μ thick, polar capsules 6·18 μ × 3.09 μ, iodinophilous vacuole 3 μ in diameter, polar filament 50 μ long.

REMARKS.—Ray (1933 *a*) reported this myxosporidian from the liver and ovary of the same fish. The Myxosporidian under report differs from any known species of *Myxobolus* both in shape and structure of the vegetative forms and the spores. It, however, shows some affinities with *M. orbiculatus* Kudo (1920) and *M. intestinalis* Kudo (1920), but differs from both of them in the shape and size of the vegetative forms and in not having folds or markings on the shell of the spores.

Myxobolus catlæ n.sp.

(Figs. 65–71)

HOST: *Catla catla* (Ham.), *Labeo rohita* (Ham.) and *Cirrhina mrigala* (Ham.). Both adult and fingerlings of these three species of fishes were heavily infected with this myxosporidian. Of fifty specimens of each species examined forty-five in *C. catla*, thirty-eight in *L. rohita* and thirty-five in *C. mrigala* were found infected.

SEAT OF INFECTION: Branchiæ.

LOCALITY: Calcutta.

VEGETATIVE FORM.—Cysts are found embeded in large numbers in the gill filaments. They are opaque white when alive and are either spherical or oval in shape. The cysts (Fig. 68) are embedded in the gill filaments. They measure 45–150 μ in largest diameter.

THE SPORE.—The shape of the spores is elongately pyriform in front view (Figs. 66, 67), with sharply pointed anterior and rounded posterior extremities. In lateral view they have the form of a spindle (Fig. 68). The shell of the spore is thin and the sutural ridge and the line could not be distinctly marked. The polar capsules are equal in size and elongated pyriform in shape. They occupy the major portion of the spore leaving for the sporoplasm a small space at their posterior end. The coiled filament is distinctly visible in fresh condition within the capsules. The sporoplasm

is generally spherical in outline containing two nuclei and a circular iodinophilous vacuole. Chromatin dots, about four in number, are seen between the polar capsules. Dimensions: length of the spore 14·5–16·5 μ, breadth of the spore 6·18 μ, thickness of the spore 5·15 μ, polar capsules 10·3–12·36 μ × 2·06–3·1 μ, polar filament 150 μ long.

REMARKS.—The parasite under report is new; it does not resemble any known species of *Myxobolus* so far described. Although it has some affinities with *M. capsulatus* Davis (1917), *M. koi* Kudo (1920) and *M. angustus* Kudo (1934) it sharply differs from them in essential features.

Some of the myxosporidian parasites are regarded as pathogenic by some workers and I have touched this aspect in a previous paper (1939). It will be interesting to mention here that infection in the fingerlings of the host fishes with *M. catlæ* proved fatal under laboratory conditions.

Thelohanellus rohitæ (Southwell and Prasad)

(Figs. 72–74)

This parasite was described by Southwell and Prasad (1919) from the gills of *Labeo rohita* which they collected from Turag river, Mirpur, in the District of Dacca, Bengal. I found the spores of this parasite infesting the gill of the same host. Southwell and Prasad's observations have been based on fixed material, and my observation given below made on fresh material are added as supplement.

HOST: *Labeo rohita.* Only one fish was found infected.

SEAT OF INFECTION: Branchiæ.

LOCALITY: Calcutta.

VEGETATIVE FORM.—Not found.

THE SPORE.—The spores (Figs. 72–74) are elongated pear-like or pyriform in shape with acutely pointed anterior and rounded posterior extremities as described by the previous authors. The size of the spores is slightly larger than that given by Southwell and Prasad (1918). The valves are thick and the sutural ridge is very prominent. The polar capsule has the same form as the spore. The former is provided with a highly coiled filament. The sporoplasm occupies the posterior portion of the spore and contains two nuclei and a spherical iodinophilous vacuole. Dimensions: length of the spore 30–33 μ, breadth of the spore 10–13 μ, polar capsule 16–20 μ × 7–8·24 μ, iodinophilous vacuole 4·5 μ in diameter, polar filament 206 μ in length.

TABLE I

Species of Fish	No. of fishes examined	No. of fishes infected	Seat of infection	Parasite	Locality
Amphipnous cuchia ..	8	5	Gall-bladder	*Chloromyxum amphipnoui* Ray	Calcutta
Anabas testudineus ..	10	2	,,	*Myxidium leiberkühni* Bütschli	,,
Catla catla ..	50	45	Branchiæ	*Myxobolus catlæ* n.sp.	,,
Cirrhina mrigala ..	50	35	,,	,,	,,
Clarius batrachus ..	12	9	Gall-bladder, liver, testes, ovary and fat bodies	*Myxobolus clarii* n.sp.	,,
Heteropneustes fossilis	12	7	Gall-bladder	*Chloromyxum amphipnoui* Ray	,,
		2	,,	*Myxidium heteropneustesi* n.sp.	
		1	,,	*Zschokkella fossilæ* n.sp.	,,
Hilsa ilisha ..	6	1	,,	*Zschokkella ilishæ* n.sp.	Bengal
Labeo rohita ..	50	38	Branchiæ	*Myxobolus catlæ* n.sp.	Calcutta
		1	,,	*Thelohanellus rohitæ* (Southwell and Prasad)	,,
Lates calcarifer ..	5	3	Gall-bladder	*Leptotheca latesi* n.sp.	Bengal
		1	,,	*Myxidium procerum* var. *calcariferi* var. n.	,,
Macrones gulio ..	5	2	,,	*Leptotheca macronesi* n.sp.	,,
Scatophagus argus ..	25	10	,,	*Certomyxa scatophagi* n.sp.	,,

Summary

1. Eight new species of myxosporidians belonging to the genera *Leptotheca, Ceratomyxa, Myxidium, Zschokkella* and *Myxobolus* have been described.

2. A new variety of *Myxidium procerum* is described.

3. A detailed description of *Chloromyxum amphipnoui* Ray is given and it is recorded from a new host.

4. *Myxidium leiberkühni* Bütschli is recorded from a new host in India.

5. *Thelohanellus rohitæ* (Southwell and Prasad) is recorded for the first time from Calcutta.

6. Infection with *Myxobolus catlæ* has been briefly discussed.

REFERENCES

Auerbach, M.* .. "Bemerkungen über Myxosporidien heimischer Süsswasserfische," *Zool. Anz.*, 1908, **32**, 456–65.

———* .. "Cnidosporidienstudien," *Ibid.*, 1910, **35**, 767–77.

———* .. "Biologische und morphologische Bemerkungen über Myxosporidien," *Zool. Anz.*, 1910 *a*, **35**, 57–63.

Awerinzew, S.* .. "Ergebnisse der Untersuchungen über parasitische Protozoen der tropischen Region Afrikas. III," *Ibid.*, 1913, **42**, 151–56.

Bhatia, B. L. .. *Sporozoa. Fauna Brit. India*, p. 336, Taylor & Francis, London, 1938.

Bütschli, O.* .. "Beiträge zur Kenntnis der Fischsporospermien," *Z. wiss. Zool.*, 1881, **35**, 629–51.

Chakravarty, M. .. "Studies on Myxosporidia from the fishes of Bengal, with a note on the myxosporidian infection in aquaria fishes," *Arch. Protistenkde*, 1939, **92**, 169–78.

——— .. "Observations on two myxosporidians *Zschokkella lissemysi* n.sp. from the gall-bladder of the tortoise, *Lissemys punctata* and *Zschokkella auerbachi* (Weill) from the gall-bladder of *Bufo melanostictus*, with a note on the genus *Zschokkella* Auerbach," *Journ. Roy. Asiat. Soc. Bengal*, 1941, **6**, 69–76.

Davis, H. S.* .. "The Myxosporidia of the Beaufort Region. A systematic and biologic study," *Bull. Bur. Fish.*, 1917, **35**, 201–43.

Fantham, H. B., Porter, A., and Richardson, L. R. "Some more Myxosporidia observed in Canadian fishes," *Parasit.*, 1940, **32**, 333–53.

Fujita, T. .. "Studies on Myxosporidia of Japan," *Journ. Coll. Agr., Hokkaido Imp. Univ.*, 1923, **10**, 191–248.

——— .. "Studies on myxosporidian infection of the crucian carp," *Japan Journ. Zool.*, 1924, **1**, 45–57.

——— .. "Studies on Myxosporidia of Japan," *Journ. Coll. Agr., Hokkaido Imp. Univ.*, 1927, **16**, 229–47.

Jameson, A. P. .. "Myxosporidia from Californian fishes," *Journ. Parasit.*, 1929, **16**, 59–68.

Kudo, R. .. "Studies on Myxosporidia. A synopsis of the genera and species of Myxosporidia," *Ill. Biol. Monogr.*, 1920, **5**, Nos. 3 and 4, 1–265.

——— .. "Histozoic Myxosporidia found in fresh-water fishes of Illinois, U.S.A." *Arch. Protistenkde.*, 1929, **65**, 364–78.

——— .. "Studies on some protozoan parasites of fishes of Illinois," *Ill. Biol. Mongor.*, 1934, **13**, 7–44.

Meglitsch, P. A. .. "On some new and known Myxosporidia of the fishes of Illinois," *Journ. Parasit.*, 1937, **23**, 467–77.

Nemeczek, A. .. "Beiträge zur Kenntnis der Myxosporidien-fauna Brasiliens," *Arch. Protistenkde.*, 1926, **54**, 137–49.

Ray, H. N. .. "Myxosporidia from India," *Proc. Indian Sci. Congr.*, 1933, Zoology, p. 259.

——— .. "Preliminary observations on Myxosporidia from India," *Curr. Sci.*, 1933 *a*, **1**, 349–50.

Schäferna, K., und Jírovec, O. .. "Zur Kenntnis des *Myxidium giardi* Cépède," *Zool. Anz.*, 1934, **105**, 266–70.

Southwell, T., and Prasad, B. .. "On some Indian Myxosporidia," *Rec. Ind. Mus.*, 1918, **15**, 344–48.

Thélohan, P.* .. "Observations sur les myxosporidies et éssai de classification de ces organismes," *Bull. soc. philom.*, 1892, **4**, 165–78.

———* .. "Recherches sur les Myxosporidies," *Bull. sc. France et Belg.*, 1895, **26**, 100–394.

* The author has not seen the original papers. The informations about the myxosporidians concerned are taken from Kudo (1920).

MASSEEELLA NARASIMHANII, A NEW SPECIES OF RUST ON *FLUEGGEA LEUCOPYRUS* WILLD.

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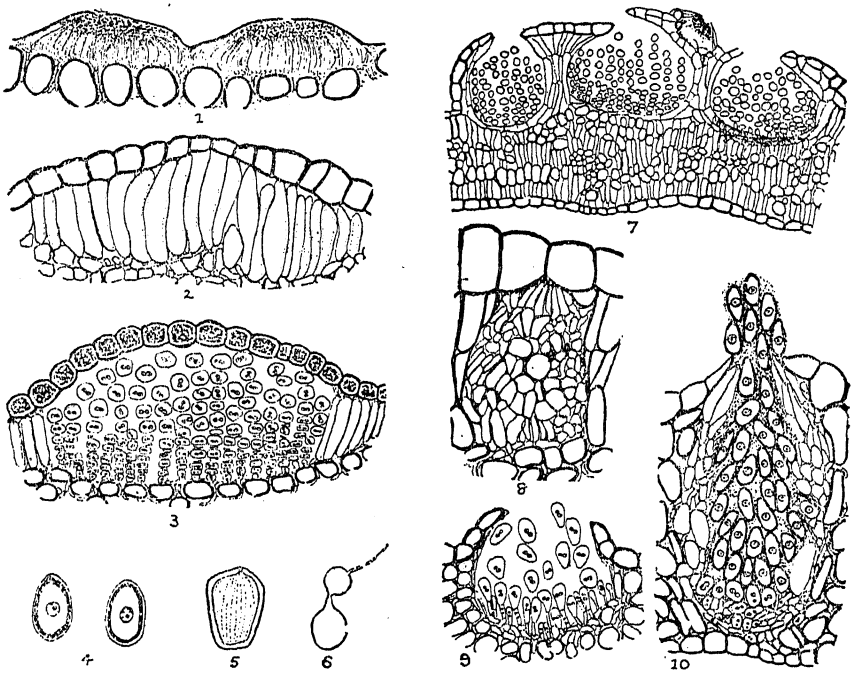
Flueggea leucopyrus Willd. is a stiff thorny shrub with ovate-orbicular leaves and greenish white fruits. Some of these plants growing near Yashavantapur, Bangalore, were heavily rusted, and a detailed study undertaken by the writer proved it to be a new species of *Masseeella*. So far three species have been recorded for the genus, *Masseeella Capparidis* (Hobson) Diet., *M. Flueggeæ* Syd. on *Flueggea virosa* Baill. in the Philippines, and *M. Breyniæ* Thirumalachar. With the exception of *Masseeella Capparidis* for which only the telial stage is known, the other two species are autœcious eu-forms. Even so the *Masseeella* on *Flueggea leucopyrus* is also an autœcious eu-form, with pycnia and æcia developing in August-September, uredia and telia in the months of October to January.

Infection spots on which pycnia and aecia are distributed, are minute, and greatly hypertrophied. They are pale yellow and do not coalesce to form a patch. Pycnia are subcuticular (Fig. 1), minute, amphigenous, hyaline and applanate. They coalesce with one another if developed in close proximity. A pore formed by the rupture of the cuticle enables the pycniospores to escape. Subcuticular pycnia have also been noticed in *Masseeella Flueggeæ* Syd. and *M. Breyniæ*.

Aecia are amphigenous and erumpent, following the pycnia in development. They are applanate, spreading, opening out irregularly. They form large chambers due to the coalescence of more than one aecium on the same infection spot (Fig. 7). The aecial initial is formed by the formation of a plectenchyma beneath the epidermis. From the upper portion of the plectenchyma, long palisade-like cells are differentiated (Fig. 2), which swell at maturity and become transformed into loose parenchymatous cells. From the basal *fertile* portion of the initial aeciospores are abstricted off in chains. The pseudoparenchymatous cells disintegrate forming a space into which the aeciospores are abstricted. The sori spread horizontally by the differentiation of more basal cells from the hymenium followed by the disintegration of the pseudoparenchymatous cells. Aeciospores are white, ovate-ellipsoid,

minutely verrucose with an indistinct germ pore, measuring 20–24 × 17 μ. The aecia are without any peridia, which however is the characteristic feature of both *Masseeella Flueggeæ* and *M. Breyniæ*, in which the peridial cells are angularly globoid, abutting on the sides, with rugose walls. In some of the



Figs. 1–6.—Fig. 1. Subcuticular pycnia of *Masseeella Narasimhanii*. ×400. Fig. 2. Elongated palisade-like cells of the aecial initial. ×320. Fig. 3. Aecium. ×320. Fig. 4. Teliospores. ×320. Fig. 5. Mature teliospore showing longitudinally striate exospore. ×320. Fig. 6. Secondary sporidium. ×400.

Figs. 7–10.—Fig. 7. Section through the infected leaf showing aecia. ×100. Fig. 8. Telial initial. ×200. Fig. 9. Uredium. ×200. Fig. 10. Mature telium with teliospores embedded in gelatinous matrix. ×200.

sections of the *Masseeella* species on *Flueggea leucopyrus*, some of the aeciospores distributed at the margin of the sorus were found to be somewhat thick-walled, and without any cell contents. They might be degenerating aeciospores or may represent evanescent peridial cells. However in the majority of aecia peridia do not occur.

The applanate spreading type of aecium (Fig. 3), bordered by palisade-like pseudoparenchymatous cells, further marked out by the lack of any definite shape and peridia distinguishes the rust from the other species. In both *Masseeella Flueggeæ* and *M. Breyniæ*, the aecia are cupulate, with a definite shape, and possessing well-developed peridia. A *Masseeella* on *Flueggea*

species collected by Rhind in Burma was sent to Kew, England, tentatively identified as *M. Flueggeæ* (Butler and Bisby, 1931). It is manifest that the exact species can be determined only after observing the aecial stages.

Uredia are hypophyllous, white, minute, aparaphysate and pulverulent (Fig. 9). The infection spots do not get hypertrophied and are often associated with pycnial and aecial pustules. Urediospores are ovate-ellipsoid, white, minutely echinulate, and stipitate. The spores are binucleate, with indistinct germ pores. They closely resemble the aeciospores. The spores are dispersed by the rupture of the epidermis. In some cases they are developed in such large numbers on the surface of the leaves, that the entire leaf surface presents a white powdery appearance.

Telia are epiphyllous and very rarely amphigenous. The sori are deeply sunk within the host tissue, almost extending up to the lower epidermis (Fig. 10). They are flask-shaped opening out by an ostiole. The teliospores are abstricted off in chains and these emerge out embedded in a gelatinous matrix secreted by the hyphæ lining the sorus. The spore tendrils measure up to 7 mm. in length. The mucilage greatly swells in water and on drying up becomes a horny mass. The telial initials are formed beneath the epidermis by the concentraction of hyphæ (Fig. 8). The teliospores are one-celled, ovate-oblong, chestnut brown, slightly angular, with an apical germ pore. The wall layer is three partite, the exospore being longitudinally striate (Fig. 5), as in *Masseeella Flueggeæ* (Cummins, 1937) and *M. Breyniæ* (Thirumalachar, 1943). Young teliospores are thin-walled, hyaline and binucleate, which later fuse to form a syncaryon (Fig. 4). The spores were germinated and stained by the method suggested by the writer (1940). The basidium is four-celled, bearing globular basidiospores on short sterigmata. Secondary sporidia (Fig. 6) have been observed.

In respect to the structure and development of the teliospores all the species of *Masseeella* so far known, show remarkably close resemblance. The telia are invariably epiphyllous, the sorus being deep seated, lined with mucilage secreting hyphæ. The teliospores of *Masseeella Flueggeæ*, *M. Breyniæ*, and in the species under study, are of same shape and possesses three wall layers, the exospore being longitudinally striate. Further, the teliospores of *M. Flueggeæ* Syd. on *Flueggea virosa* and those of *Masseeella* sp. on *Flueggea leucopyrus* closely resemble each other as regards the size and shape. But the urediospores and æciospores differ in spore measurements. The urediospores of *M. Flueggeæ* measure 20–26 × 16–20 μ, whereas those of the present rust are smaller in size measuring 12·7–25 × 12·7–16 μ. On the other hand the aeciospores are larger in the latter form than in

M. Flueggeæ. They measure 13–16 × 16–23 μ in *M. Flueggeæ* as against 20–24 × 17 μ in the *Masseeella* species on *Flueggea leucopyrus*. In addition, the lack of peridium and absence of cupulate aecia in the *Masseeella* on *Flueggea leucopyrus* and other characters necessitate the erection of a separate species for its accommodation. The name *Masseeellá Narasimhanii* is proposed in honour of Mr. M. J. Narasimhan, Director of Agriculture, Mysore State. The telial stages of this rust were also collected by Md. Taslim on 26–11–1936 at New Delhi on the same host, and the rust has evidently a wide distribution.

Masseeella Narasimhanii Spec. Nov.

Pycnia subcuticularia, complures, plus minus densissime aggregati, maculis contagium tumidulus. Aecia subepidermalia, irregularitier erumpentia, aliquanto applanata, flave albida, peridio evanidus vel absens; aeciosporæ alba, subglobosæ ovatæ vel ellipsoidæ, minutissime verrucosæ, poris germ. indistinctæ, magnitudinis 20–24 × 17 μ. Urediosori hypophylli, maculis obsoletis, subepidermicis, aparaphysatis; urediosporæ ovatæ vel ellipsoidæ, verrucosæ, membrana hyalina, solitarie ortæ, poris germ. obscuris. Telia epiphylla, rarissime singulæ, etiam hypophylla, profundi immersi, sporas in cirras filiformes, copiosæ aggregati, vel solitarie, nigro-brunneos, 3–7 mm. longos; teliosporæ oblong ellipsoidæ, vel fusiformæ, sessiles, leniter angulatæ, castanneo-brunnæ, spora membrana tripartitus, exosporæ subtilissime costatæ longitrosum, magnitudinis 22–31 × 14·5–17 μ., in massa mucosa sitæ, poris germ apicali, sporæ statim germinantes, promycelio externo, typice 4-cellulari; sporidis globosus, tenuiter membrana, secundario sporidiis formant.

Hab.—In vivis foliis *Flueggea Leucopyrus* Willd., Yashavantapur, Bangalore, 15—9—1942, leg. M. J. Thirumalachar (Type), New Delhi, 26—11—1936. leg. Md. Taslim. Type deposited in the Herb. Crypt. Ind. Orient., New Delhi.

Pycnia subcuticular, densely aggregated, hyaline, on swollen infection spots. Aecia subepidermal, applanate, yellowish white, opening irregularly, peridium evanescent or absent, aeciospores white, subglobose, ovate-ellipsoid, minutely verrucose, germ pores indistinct, measuring 20–24 × 17 μ. Uredial infections not causing hypertrophy, uredia white, hypophyllous, subepidermal and aparaphysate; urediospores ovate-ellipsoid, minutely verrucose, single indistinct germ pore, measuring 12·7–25 × 12·7–16 μ. Telia epiphyllous, rarely hypophyllous, in solitary curly hair-like columns, or densely aggregated, horny, teliosorus conical, deeply sunk, single-celled

spores abstricted in succession, which emerge out embedded in gelatinou matrix secreted by the hyphæ lining the sorus; teliospores spherical, fusifom oblong-ellipsoid, slightly angular, chestnut brown, wall layer three partit longitudinally striate, with apical germ pore, spores measuring 22–31 14·5–17 μ. Sporidia spherical, secondary sporidia also formed.

Hab.—On living leaves of *Flueggea leucopyrus* Willd. Yashavantapu Bangalore, 15—9—1942, leg. M. J. Thirumalachar (Type), and New Delh 26—11—1936, leg. Md. Taslim. Type deposited in the Herb. Crypt. Inc Orient., New Delhi.

In conclusion the wirter wishes to acknowledge his indebtedness t Dr. B. B. Mundkur, Inperial Agricultural Research Institute, New Delhi, fo critically going through the manuscript and valuable suggestions, and t Dr. L. N. Rao, Professor of Botany, University of Mysore, for helpful sugge tions and encouragement.

LITERATURE CITED

1. Butler, E. J., and Bisby, G. R. "Fungi of India," *Imp. Council Agri. Res. Scien. Monograp* 1931, No. 1, 51.
2. Cummins, G. B. .. "Studies in the Uredinales of the Philippines," *Ann. Myc Berlin,* 1937, **35,** 130.
3. Sydow, H., and Petrak, F. .. "Micromycetes Philippinensis," *ibid.*, 1928, **26,** 424.
4. Thirumalachar, M. J. .. "A method for germinating and staining teleutospores *Jour. Ind. Bot. Soc.*, 1940, **19,** 71–75.
5. ———— .. . "*Masseeella Breyniæ*, A New species of Rust," 1943 (In Pres

TENT CATERPILLAR (*MALOCOSOMA INDICA* WLK.) IN THE SIMLA HILLS

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Introduction.—During the course of an entomological survey of the orchards in the Kotgarh ilaqa (Simla Hills) in July-August, 1940 a large number of egg-masses, resembling those of the notorious Tent Caterpillar of America, were found deposited on branches and twigs of a number of apple and a few pear, plants. The twigs bearing these egg-masses were brought to the laboratory at Kotgarh and kept under observation. They hatched on the 20th March, 1941 and the adults which emerged from them in due course were identified as *Malocosoma indica* Wlk. by a Systematist of the Imperial Agricultural Research Institute, New Delhi.

This article embodies the results of observations made on *M. indica* Wlk. since its first discovery.

Food plants.—The pest has been recorded as occurring on apple pear, apricot, walnut, " shaigal " (*Pyrus* sp.), and " ban " (*Quercus incana*) and of these apple generally, and pear occasionally, suffer the most from its ravages.

Distribution in the Simla Hills.—The pest has so far been collected from Aina, Bhareri, Himtala, Kotgarh proper, Pomlai, Rhoga and Thanedhar in the Kotgarh ilaqa; Kudiali and Matiana on the Hindustan-Tibet Road (between Kotgarh and Simla); Cheriot Nal, Chharabra, Gahan, Mashobra and Sanjauli near about Simla; and from Kasauli.

Description of various stages: Egg.—About 1 mm. long, elongate, thimble-shaped and shining.

Full-grown larva.—Length 40 to 45 mm., breadth 4·5 to 5·5 mm. Head and dorsum black. Dorsum ornamented with (*a*) a broad greyish line (dotted with black) which extends antero-posteriorly medially and (*b*) four lines of crimson coloured dots, two on either side of (*a*). Legs black, prolegs light brown. Body cylindrical with a lateral tuft of white hairs on each segment. Dorsum sparingly clothed in fine black bristles arising from tubercles ; those on the last two abdominal segments being more numerous.

Last abdominal segment triangular and furnished with two lobe-like appendages at posterior end.

Pupa.—18 to 21 mm. long, 6 to 7 mm. broad, brown to dark brown, more or less smooth.

Adult: Male:—Wing expanse 29–32 mm. Light reddish in colour. Forewings traversed by two oblique, broad, whitish stripes enclosing whitish area in between. Antennæ thick, many jointed and bipectinate.

Female.—Wing expanse 35–37 mm. Light brown, stripes on the fore-wings rather less prominent. Antennæ similar to those of the male.

Life-history.—The pest hibernates in the egg-stage. Females lay eggs as broad bands round branches of plants. Each band, which consists of 200 to 400 eggs, is covered with a protective layer of dark brown gluey substance. When buds appear (about the middle of March) these eggs hatch out (Table I).

TABLE I. *Duration of egg-stage of* Malocosoma indica *Wlk. at Kotgarh*

Eggs laid on	Eggs hatched on	Duration of egg stage	
		Months	Days
22-5-1941	18-3-1942	9	27
30-5-1941	18-3-1942	9	19
4-6-1941	18-3-1942	9	14

Thus the egg-stage lasts for about 9 to 10 months.

The larvæ live gregariously. Soon after hatching they spin a silken nest at a convenient and sheltered place on the plant. The nest, or the tent as it is called, is at first small but it gradually increases in size as the caterpillars grow bigger until in some cases it may nearly be 1 to 1½ ft. in length. In cases of serious infestation there may be as many as 18 to 25 such ' tents ' on a single plant. These ' tents ' render a plant unsightly. The caterpillars spend the day in the ' tent'; they feed on the leaves of the plant at night. When full-fed the caterpillars seek out a protected place where they spin their cocoons. Duration of the larval stage varies from 39 to 68 days at Kotgarh (Table II).

TABLE II. *Duration of larval stage of* Malocosoma indica *Wlk. at Kotgarh*

No.	Eggs hatched on	Larvæ pupated on	Duration of larval stage (days)
1	20-3-1941	28-4-1941	39
2	20-3-1941	3-5-1941	44
3	20-3-1941	20-5-1941	61
4	20-3-1941	27-5-1941	68
5	18-3-1942	9-5-1942	52
6	18-3-1942	13-5-1942	56
7	18-3-1942	17-5-1942	60

The oval white cocoons, which were about 1" in length, were found to be made of compactly woven silk. Pupal stage was found to last for 8 to 22 days (Table III).

TABLE III. *Duration of pupal stage of* Malocosoma indica *Wlk. at Kotgarh*

No.	Larvæ pupated on	Moths emerged on	Duration of pupage stage (days)
1	30-4-1941	8-5-1941	14
2	8-5-1941	19-5-1941	11
3	16-5-1941	2-6-1941	17
4	8-5-1942	30-5-1942	22
5	1-5-1942	20-5-1942	19
6	8-5-1942	29-5-1942	21

Preoviposition period.—Preoviposition period was found to be 1 to 3 days.

TABLE IV. *Preoviposition period in* Malocosoma indica *Wlk. at Kotgarh*

No.	Pairs mated on	Eggs laid on	Preoviposition period (days)
1	20-5-1942	22-5-1942	2
2	30-5-1942	31-5-1942	1
3	1-6-1942	4-6-1942	3

In captivity a female moth lived for 3 to 5 days and a male 4 to 6 days.

Proportion of sexes.—Table IV below gives the proportion of sexes among the moths which emerged in the laboratory at Kotgarh during 1941 and 1942.

TABLE V. *Proportion of sexes*

Year	Percentage of males	Percentage of females
1941	56·2	43·8
1942	61·5	38·5

Seasonal history and duration of life-cycle.—There is only one generation of the pest in a year. Over-wintered eggs started hatching from the middle of March and continued till May-June. Emergence of moths began in the 3rd week of May and lasted till the beginning of June.

Table VI below gives the duration of life-cycle.

TABLE VI. *Duration of total life-cycle of* Malocosoma indica *Wlk. at Kotgarh*

No.	Eggs laid on	Eggs hatched on	Larvæ pupated on	Adults emerged on	Duration of total life-cycle
1	22–5–1941	18–3–1942	1–5–1942	20–5–1942	One year
2	4–6–1941	18–3–1942	16–5–1942	1–6–1942	One year

Nature and extent of damage.—The caterpillars feed on leaves gregariously. In cases of serious infestation the entire plant was found to be almost completely defoliated, mid rib and other harder veins of the leaves only were left behind. Such plants did not bear any fruit. In the absence of leaves young caterpillars were noticed to feed on tender bark. In years of serious infestation as many as 40 to 50% of the apple plants in an orchard were found to be infested.

Control.—1. As is evident from the life-history details, egg-stage lasts for about 9 to 10 months. Egg clusters which are laid round branches and twigs are the easiest to destroy, the most convenient time for the purpose being December-January. When pruning, branches and twigs with egg clusters, should be carefully searched out and collected and either burnt or buried about 1 foot deep in the soil.

2. The caterpillars should be killed by rubbing the 'tents' with rags dipped in kerosene oil and tied at the end of a pole. This control gave the best results when carried out from 12 noon to 3 p.m. on clear sunny days.

An open vessel containing water with a film of kerosene oil on surface was placed on the ground just underneath the 'tent' when applying the 'kerosenised rags' so that any larvæ which fell down at the touch of the 'rag' were killed in the treated water. The cost of operation was worked out at As. 4 per 10–15 tents.

NITROGEN REQUIREMENTS AND VITAMIN DEFICIENCIES OF *PHYTOPHTHORA PHASEOLI* THAXTER

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IT has been well established that the source of nitrogen, in the cultural nutrition of fungi, is an important limiting factor (Robbins, 1937, Leonian and Lilly, 1938), but for some even the proper source of nitrogen often may be ineffective without the presence of growth supplements. Robbins (1938), who studied the nutrition of some species of *Phytophthora*, found that they apparently required for their growth an external supply of thiamin. Leonian (1925, p. 448) has reported that *Phytophthora phaseoli* grows very slowly on the two media used by him. One contained malt extract (dry), KH_2PO_4, $MgSO_4$ and Bacto agar, while the other was made up of nucleinic acid, KH_2PO_4, $MgSO_4$, dextrose and Bacto agar. The latter medium was used without agar also. When he added a few c.c. of lima bean infusion to the media, the fungus showed rapid growth. His results indicate that *P. phaseoli* suffers from a deficiency of some growth supplements not amply represented in the original media used by him.

The present paper deals with the nitrogen requirements and vitamin deficiencies of *Phytophthora phaseoli* Thaxter.

For a comprehensive review on these subjects the reader is referred to Robbins (1937) and Robbins and Kavanagh (1942).

Material and Methods

The culture of *Phytophthora phaseoli* Thaxter was obtained from Centraal Bureau voor Shimmelcultures, Baarn, Holland.

The methods and technique employed in this investigation were essentially the same as described in previous papers (Saksena, 1941 *a* and *b*; Saksena and Bhargava, 1941).

Unless otherwise indicated the fungus was grown in 150 ml. Erlenmeyer Pyrex flasks containing 15 c.c. of a basal medium, which will afterwards be referred to as medium M, consisting of 0·5 gm. each of KH_2PO_4, $MgCl_2 \cdot 6H_2O$ and K_2SO_4, 5·0 gm. of pure dextrose (dextrosol of Corn Products Co.)

and 1 litre of distilled water. Magnesium chloride was prepared by the action of pure hydrochloric acid on the clean magnesium ribbon; the commercial guaranteed reagent ($MgCl_2$. $6H_2O$) was found to be of no use in these experiments, because it contained traces of ammonia. Asparagin and alanine were purified by repeated precipitations with alcohol.

Only guaranteed reagents (either from Merck or from British Drug House) were used. Thiamin used was Merck's vitamin B_1 and the yeast extract was a Difco product. Lima bean infusion was obtained by steaming 2 gm. of lima beans (*Phaseolus lunatus**) in 100 c.c. of water for one hour, while lentil extract was prepared according to the process used by Buston and Pramanik (1931). Casein was brought into solution by dissolving 1 gm. of casein in 8 c.c. of N/10 NaOH, then adjusting the reaction with HCl.

The stock cultures were maintained on oatmeal agar. All cultures were grown in triplicate. The range of temperature during the experiments was 20°–22°C. and the pH of the media was adjusted to 5. Generally the incubation period was of 15 days' duration. Throughout the experiments only Pyrex glass ware was used.

In order to decrease the effect of whatever vitamins might have been present in the original inoculum coming from cultures grown on oatmeal agar, subcultures from the growth in the first passage in liquid medium were made into corresponding medium for a second passage. A particular medium was thought suitable only when the fungus grew on it by subsequent transfers.

Experimental

Nitrogen requirements.

Series I. To the basal medium M, the following inorganic and organic nitrogen-containing substances were added singly in 0·1 per cent. concentration. The growth of the fungus in a medium indicated the utilisation of the particular mitrogen compound. Medium M served as a control.

Inorganic sources.—Ammonium nitrate, sodium nitrate and sodium nitrite.

Organic sources.—Amino acids: Glycine, *d*-alanine, purified *d*-alanine, *d*-valin, *l*-leucin, *d*-arginine, *d*-lysin, *l*-asparatic acid, *d*-glutamic acid, asparagin, purified asparagin, *l*-phenyl-alanine, tyrosin, histidin, tryptophane, proline, cystin and cystein hydrochloride. Amide: Acetamide. Amines: Urea and trihydroxy-triethylamine. Proteins: peptone, hydrolysed peptone, purified casein and butter milk.

* This is the host of *P. phaseoli.*

It was found that *P. phaseoli* grew only in media containing *d*-alanine, peptone, hydrolysed peptone and butter milk, while it did not respond positively to other media.

Series II.—Since the fungus grew in *d*-alanine and not in purified *d*-alanine, it was thought that in *d*-alanine was some unknown substance essential for its growth and that it got removed during the process of purification. The various washings obtained during purification were collected and evaporated to dryness. A little of this substance was added to medium M, containing purified *d*-alanine. Medium M with purified *d*-alanine served as a control. The two media were then inoculated with the fungus, which grew only in the former.

Relation to Vitamins.

The results obtained above indicated that the fungus required some growth substances for its growth and that they were present in *d*-alanine, peptone, hydrolysed peptone and butter milk. Since peptone has been found to have a small amount of thiamin or its intermediates (Robbins and Schmidt, 1938) the following procedure was adopted.

Series III.—The media used in the experiments described under Series I were inoculated with spore suspensions of *Phycomyces blakesleeanus* which requires thiamin or its intermediates for its growth (Schopfer, 1934). The results obtained were all negative except in the case of media containing *d*-alanine, asparagin, peptone, hydrolysed peptone and butter milk.

Series IV.—To the various media used in Series I was added thiamin (5 international units per 25 c.c. of the medium). They were then inoculated with *Phytophthora phaseoli.* Good growth was obtained in media containing *d*-alanine, purified *d*-alanine, peptone, hydrolysed peptone, purified casein and butter milk.

Series V.—The basal medium M and other media used in Series I were supplemented with the following products of natural origin, in the concentration noted against each:

(*a*) lentil extract	0·02%
(*b*) yeast extract	0·01%
(*c*) lima bean infusion	1 c.c. per 10 c.c. of the medium.

These were then inoculated with *P. phaseoli.* The fungus made good growth in all the cases except where the source of nitrogen was NH_4NO_3.

Toxic effect of ammonium salts.

Series VI (*a*).—From the experiments reported under Series V, it appeared that NH_4NO_3 had some toxic effect on the growth of the fungus. To ascertain, if it was so, the fungus was grown on a richer medium (Leonian, 1930, p. 673) containing proteose peptone 2 gm.; KH_2PO_4 0·5 gm., $MgSO_4$ 0·5 gm., succinic acid 0·2 gm., dextrose 5 gm. and water 1,000 c.c. supplemented with NH_4NO_3 and various growth substances. The results obtained are given in Table I, and shown in Fig. 1.

TABLE I

Dry weight of mycelium (in milligrams) of the fungus colony grown for 30 *days*

Medium	Dry wt.
1. Leonian's medium (under Series VI *a*)	7
2. Medium 1 + NH_4NO_3 0·2%	×
3. Medium 1 + thiamin (5 units per 25 c.c.)	15
4. Medium 3 + NH_4NO_3 0·2%	×
5. Medium 3 + ascorbic acid (1 unit per 25 c.c.)	15·5
6. Medium 5 + NH_4NO_3 0·2%	×
7. Medium 1 + lentil extract 0·02%	19
8. Medium 7 + NH_4NO_3 0·2%	×
9. Medium 1 + bean infusion (1 c.c. per 10 c.c.)	30
10. Medium 9 + NH_4NO_3 0·2%	19
11. Medium 1 + yeast extract 0·01%	18
12. Medium 11 + NH_4NO_3 0·2%	×

× Indicates absence of growth.

Series VI *b*.—To know exactly whether the ammonium ion or the nitrate ion is responsible for the toxicity Leonian's medium used in Series VI *a* was supplemented with thiamin (5 units per 25 c.c.), and NH_4Cl, $(NH_4)_2SO_4$ and $NaNO_3$ were added to it singly in 0·2% concentration. The fungus showed growth on Leonian's medium supplemented with thiamin and on the medium containing $NaNO_3$ while it did not grow on those to which NH_4Cl or $(NH_4)_2SO_4$ had been added.

Discussion and Conclusions

Earlier work of Leonian (1925, 1930) on the nutrition of the genus *Phytophthora* furnishes little information on the nutritional requirements of *Phytophthora phaseoli*. The inability of *P. phaseoli* to grow on nutrient media containing inorganic nitrogen (Series I) clearly indicates that probably it is one of those fungi which do not assimilate ammonia or nitrate but require some organic nitrogen. The positive reaction which the organism shows

with *d*-alanine and peptone justifies its being placed in the 'organic nitrogen organisms' classified and placed separately by Robbins (1937). Kincaid (Robbins 1937, p. 244) found that *Blepharospora cambivora, Diplodia Zeæ, Endomyces Magnusii, Phycomyces nitens,* thirteen species of *Phytophthora, Sphæronema fimbriatum* did not grow with either nitrate or ammonia as the source of nitrogen and dextrose as the source of carbon but grew with peptone. Similarly *Euglena deses* and *E. pisci-formis* do not assimilate nitrate or ammonia but require amino acids or peptone (Dusi, 1933).

But the relation of organisms to organic nitrogen is generally complicated by the possible contamination of organic nitrogen compounds with the presence of accessory growth factors, which are not generally found in pure inorganic salts. In the present case, *d*-alanine, peptone, hydrolysed peptone and butter milk are found to contain traces of thiamin or its intermediates (Series III). Since lentil extract (Hawker, 1936, p. 703) and yeast extract (Leonian and Lilly, 1938) in addition to some of the known vitamins contain amino acids and other organic nitrogen compounds, it is clear that they supply the necessary source of organic nitrogen in addition to thiamin (Series V).

That thiamin is also essential for the growth of *P. phaseoli* is shown by the fact that it fails to grow on medium M containing purified *d*-alanine but shows good growth in the presence of *d*-alanine (Series II), which has been demonstrated to contain thiamin or its intermediates (Series III). This conclusion is also supported by the experiment carried under Series IV where the addition of thiamin to medium M containing purified *d*-alanine or purified casein induces the growth of the organism. It may be noted here that the fungus grows well on medium M containing lime bean infusion (Series V). This shows that lima bean infusion is a source both of a suitable organic nitrogen and growth supplement for the organism.

Since peptone is a complex mixture containing minerals, amino acids, several vitamins as well as other nitrogen compounds, no important conclusion as regards nitrogen requirements of the fungus can be derived from its use. Similar is the case with lentil extract, yeast extract and lima bean infusion. The most important result is the presence of growth on medium M containing purified *d*-alanine supplemented with thiamin (Series IV). This shows that *P. phaseoli* requires a special amino acid as source of nitrogen for its growth and that it suffers from thiamin deficiency. Both amino acids and thiamin are considered necessary by Leonian and Lilly (1938) for *Coprinus lagopus, Chætocladium Brefeldii, Nyctalis asterophora, Pilaira moreaui* and *Pleurotus corticatus* also.

The other result to be interpreted is the failure of the organism to grow on a medium containing any ammonium salt. It is clear from the results of the experiments carried under Series VII *a* and *b* that ammonium salts have a toxic effect on its growth. Streets (1937) found ammonium compounds toxic to the root rot fungus when supplied in sufficient concentration. According to Blank (1941) and Blank and Talley (1941) poor growth with the ammonium salts can be attributed either to the rapid development of critical acidity or to a reduction in the availability of the trace elements. The media used by the authors do not contain high concentrations of ammonia, the salts of which are added in 0·2% concentration. There is no lack of trace elements in them since the fungus grows well in Leonian's medium with or without the addition of thiamin, yeast extract, lentil extract and bean infusion (Series VII *a*).

That a very acid condition of the media inhibits the fungal growth cannot be a fact in this case since the pH of the media containing ammonium salts, on which the fungus shows no growth, is the same, *i.e.*, pH 5 as that of the media without ammonium salts, on which the fungus grows. This conclusion is supported by the result of an experiment, not reported in the foregoing pages, that the fungus grows in 1% Difco bacto peptone solution, while it fails to do so when NH_4NO_3 is added to this solution, the pH in both the cases being 6·9.

It is evident that none of these factors are responsible for the absence of growth on the media supplemented with ammonium salts. Our results indicate that ammonium ion has a toxic effect on the growth of the fungus, but this toxicity may be due to the presence of some growth inhibitors in the purest ammonium salts obtained from the manufacturers (*i.e.*, *Proanalysi* of Merck's and Analar of British Drug House). It may be mentioned here that in a large number of fungi which have been or are being investigated in this laboratory, the same ammonium salts were used and none of them has so far been found toxic for any other fungus. The nature of toxicity will be dealt with in a subsequent note.

Summary

Phytophthora phaseoli Thaxter is unable to grow on a medium containing mineral salts, dextrose and inorganic nitrogen but requires for its growth a special amino acid (*d*-alanine) supplemented with thiamin. Other substances found to be suitable as nitrogen sources are peptone, hydrolysed peptone, casein, buttermilk, lentil extract, yeast extract and lima bean infusion, all of which, excepting casein, also supply the necessary growth substance.

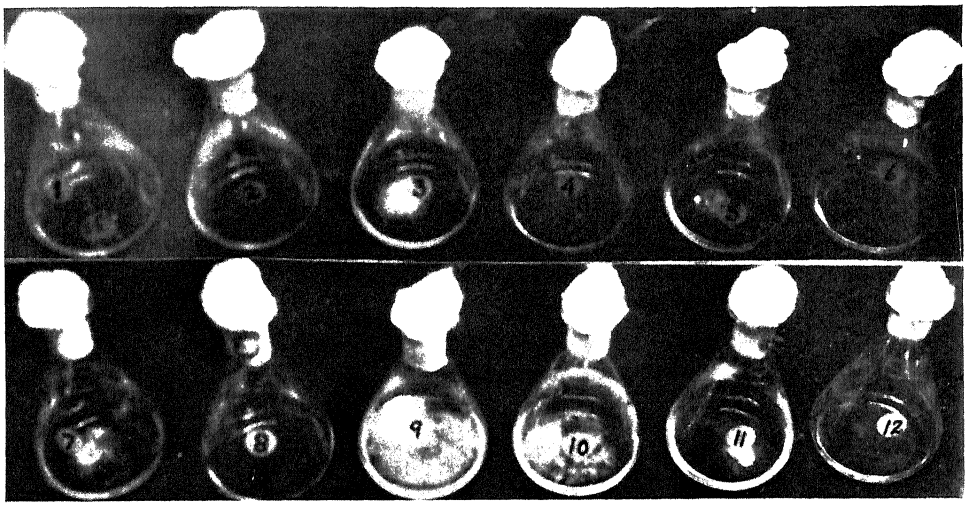

FIG. 1. Growth of *Phytophthora phaseoli* on various media used in Series VI *a*

(1) Leonian's medium. (2) Medium 1 + NH_4NO_3. (3) Medium 1 + Thiamin. (4) Medium 3 + NH_4NO_3. (5) Medium 3 + Ascorbic acid. (6) Medium 5 + NH_4NO_3. (7) Medium 1 + lentil extract. (8) Medium 7 + NH_4NO_3. (9) Medium 1 + bean infusion. (10) Medium 9 + NH_4NO_3. (11) Medium 1 + yeast extract. (12) Medium 11 + NH_4NO_3.

The fungus suffers from thiamin deficiency. Ammonium ion is found to be toxic for the growth of the fungus.

LITERATURE CITED

Blank, L. M. .. *Jour. Agr. Res.* (U.S.), 1941, **62,** 129–59.

——— and Talley, P. J. .. *Phytopath.*, 1941, **31,** 926–35.

Buston, H. W., and Pramanik, B. N. *Biochem. Jour.*, 1931, **25,** 1656–70.

Dusi, H. .. *Ann. Inst. Pasteur.*, 1933, **50,** 840–89.

Hawker, L. E. .. *Ann. Bot.*, 1936, **50,** 699–717.

Leonian, L. H. .. *Amer. Jour. Bot.*, 1925, **12,** 444–98.

——— .. *Ibid.*, 1930, **17,** 671–77.

——— and Lilly, V. G. .. *Phytopath.*, 1938, **28,** 531–48.

Robbins, W. J. .. *Amer. Jour. Bot.*, 1937, **24,** 243–50.

——— .. *Bull. Torrey Bot. Club*, 1938, **65,** 267.

——— and Kavanagh, V. .. *Bot. Rev.*, 1942, **8,** 411–71.

——— and Schmidt, M. B. .. *Bot. Gaz.*, 1938, **99,** 761–728.

Saksena, R. K. .. *Proc. Ind. Acad. Sci.*, 1941*a*, **14,** 141–48.

——— .. *Jour. Ind. Bot. Soc.*, 1941*b*, **20,** 183–89.

——— and Bhargava, K. S. .. *Proc. Nat. Acad. Sci. India*, 1941, **11,** 27–40.

Schopfer, W. H. .. *Arch. Mikrobiol.*, 1934, **5,** 511–49.

Streets, R. B. .. *Ariz. Agr. Exp. Stat. Tech. Bull.*, 1937, **71,** 299–410.

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VISCO-ELASTIC PROPERTIES AND CONTRACTION OF UNSTRIATED MUSCLE

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THERE is a close resemblance between the effects of ions on the protoplasmic viscosity of simple organisms and plants on the one hand and their effects on the viscosity of unstriated muscle on the other. The viscosity of the protoplasm in these simple organisms can be measured by standard methods (for literature, see Heilbrunn, 1937), but the viscosity of unstriated muscle is measured by comparing it to known viscous-elastic systems (Winton, 1930; Singh, 1938 *c*, 1942 *a*, 1943 *a*, *b*); the similarity of the effects on the simple organisms and the muscle gives an insight into the structure of the latter, and further establishes the validity of the indirect methods used for determining its viscosity.

Methods

I have used three methods for determining the viscosity of unstriated muscle. The first method is a qualitative one used by Winton (1930); an increase in the rate of change of length is presumed to be due to decrease of viscosity. In the second method, the viscosity has been calculated from the gradient of the linear phase; the rate of extension will be directly proportional to the extending force, and inversely to the viscosity. The extending force is opposed by tone of the muscle. In the third method viscosity has been calculated from the exponential curve obtained by subtracting the linear phase from the time extension curve of plain muscle.

The validity of the last method is derived from the experiments of Hill (1936) on the extension of tetanised striated muscle and of Singh (1938 *c*). If the load is great enough, a tetanised striated muscle does not stretch exponentially but gives way before the load, owing to "collapse of the contractile mechanism". The curves then resemble those of unstriated muscle. It is obvious, that had the contractile mechanism not given way, then the muscle would have stretched exponentially. If from the actual curve, the rate of collapse be subtracted, then an exponential curve would be obtained, which would have been produced if the muscle had not yielded before the load.

In unstriated muscle, the linear phase is due to the " collapse of tone ", and if the linear phase is subtracted from the time extension curve, the resulting exponential curve would represent the extension of the muscle, had the tone " not collapsed ". The determination of viscosity, then, by the method of Bouckaert, Capellan and de Blende (1930) is valid.

These three methods, qualitatively give identical results, with one or two discrepancies, such as the effect of magnesium. The fact however, that the results obtained by the last method show close resemblance to those obtained in simple organisms by direct methods, points to some justification in use of the method, and the results obtained thereby are described.

Results

Sodium and potassium increase and magnesium and calcium decrease the viscosity of the interior protoplasm of the sea urchin Arbacia, of the Protozoa Stentor and Amœba and of the alga Spirogyra (Heilbrunn, 1923; Weber, 1924; Heilbrunn and Daugherty, 1931). Sodium chloride and small concentrations of potassium (0·1 M KCl), calcium (0·01-0·02 M $CaCl_2$), magnesium (0·05 M $MgCl_2$) have similar effects on the viscosity of *Mytilus* muscle (Singh, 1938 *c*, 1942 *a*, 1943 *a*).

In the cortical protoplasm of the amœba and the protoplasm of the sea urchin egg, calcium causes a pronounced stiffening of the cortical gel, and this effect is antagonised by sodium, potassium and magnesium; in the case of the amœba potassium has the strongest liquefying effect, magnesium the next and sodium has the least action (Heilbrunn and Daugherty, 1932). Potassium loses its liquefying action in acid solution (Heilbrunn and Daugherty, 1934). The above is precisely the action of large concentrations of these ions on the viscosity of *Mytilus* muscle. If the sodium chloride of the saline is replaced with calcium, magnesium, or potassium chlorides the viscosity is increased in the order Ca> Na> Mg> K. Indeed potassium is the most powerful agent I have known, that decreases the viscosity; the effect of potassium is completely counteracted if the pH of saline is reduced to 5–4·4.

Barth (1929) found that when the cells were immersed in acid solutions at a pH of 5 of below, the protoplasm was coagulated. The effect was more rapidly produced when the acids were dissolved in sodium chloride solution instead of sea water. In *Mytilus* muscle too, viscosity is increased more in acid solutions of sodium chloride than in acid sea-water or in the presence of calcium. Barth found also that alkalies tend to liquefy the protoplasm of sea urchin eggs. In amœba, acids increase the viscosity both of the interior

protoplasm and of the outer cortical plasmagel. On the other hand, alkalies increase the viscosity of the interior protoplasm, but liquefy the plasmagel (Heilbrunn, 1937). In *Mytilus* muscle too acids increase the viscosity.

Relation between experiments on isolated myosin and isolated muscle.—Astbury and Dickinson (1940) have shown that isolated strips of myosin from *Mytilus* muscle show thermal contraction below 40° C. It is interesting to note that living muscle also begins to show increased tone at this temperature (Rao and Singh, 1940; Singh 1942 *b*). The above authors also found that water made myosin contract. Unstriated muscle also shows increase in tone when immersed in hypotonic solutions (Singh, 1939 *b*). *Mytilus* muscle actually contracts when immersed in distilled water. The effect of alkalies is to increase the contraction of myosin; in actual muscle, tone is increased in alkaline solutions (Singh, 1938 *b*; 1939 *b*; 1942 *b*).

Reactions of dying muscles.—It is interesting to note the condition in which the muscle dies. In isotonic solutions of sodium chloride, sodium bromide, sodium nitrate, sodium cyanide, barium chloride, and calcium chloride *Mytilus* muscle dies in a contracted condition. In sodium iodide, sodium thiocyanate, strontium chloride and magnesium chloride it dies in a relaxed condition. Calcium (0·01–0·02 M $CaCl_2$) counteracts the effect of sodium chloride. These observations show the direct action of ions on the myosin muscles soaked for 16 hours isotonically.

Partial contraction of unstriated muscle.—It was found by Fletcher (1937) and independently by me that sections of *Mytilus* muscle showed two kinds of fibres. It was found by me that if the muscle was treated with barium chloride, then the diameter of the fibres became uniform. This shows that partial contraction of plain muscle is also a function of the number of contracting fibres. It has been shown by Fletcher that the fibres of *Mytilus* muscle run from end to end. As sodium chloride can cause local contracture of the muscle, it shows that the portions of fibres can contract, and that the excitability of the fibre varies in different places. Also the excitability of individual fibres in a muscle varies, as shown by the above observations.

Discussion

The similarity of the effects of ions on the protoplasmic viscosity of simple organisms and unstriated muscle suggest that unstriated muscle fibres at least partly consist of undifferentiated protoplasm like that of the amœba. The fact that isolated myosin is affected by temperature, alkalies and distilled water in a similar way as isolated living muscle suggests, that the contractile element consists of myosin.

Unstriated muscle fibres, therefore, consist of (*a*) viscous element, (*b*) a non-viscous contractile element. This view is in agreement with (1) the differential action of drugs, (2) the visco-elastic properties, and (3) the histological picture of the muscle. Thus calcium decreases the viscosity of both the guinea pig uterus and *Mytilus* muscle, but causes contraction of the former and relaxation of the latter. Similarly hydrogen ions increase the viscosity of *Mytilus* muscle, but may cause contraction or relaxation. Viscous elastic properties show a contractile and a viscous element. Lastly histologists have described plain muscle fibres as consisting of fibrils embedded in sarcoplasm. The contractile fibrils may be disseminated throughout the sarcoplasm or may be collected into a perepheral zone (Roskin, 1925).

The observations on the similarity of the responses of myosin and muscle are interesting in view of their bearing on the nature of muscular contraction. A substance may act directly on the contractile mechanism of the muscle, or indirectly through the excitability system. If any agency affects myosin and muscle identically, then it acts on the contractile mechanism, though it may also affect the excitability mechanism; a rise in temperature will directly affect the contractile mechanism.

Contractioin of myosin and muscle under similar conditions and the responses of dying muscles, suggest the possibility of muscular contraction without the use of oxygen consumption, as is well known to occur in unstriated muscle. It is quite clear that the contraction of muscle can occur without the usage of oxygen, and is a property of the myosin molecules.

The question then arises as to why in one kind of contraction there is increase in oxygen consumption and not in the other. It may be that the former contraction is maintained by an unstable metabolite, which by combining with myosin, induces attraction between its component parts, while in unstriated muscle, after the latter is contracted, such a metabolite is not necessary; this latter property may be related to the greater extent which unstriated muscle can contract when compared with striated muscle. Such contraction may result in parts of the molecule coming close enough so that attractive forces come into play without any intervening agency; or the contraction may be due to a stable metabolite.

The catch mechanism of the plain muscle may be a function of the viscosity, or the property of the myosin molecules themselves. Whichever it is, energy is required to set as well as undo the catch; so this explains why plain muscle requires oxygen both for contraction as well as relaxation (Rao and Singh, 1940). The action of sodium chloride on dying muscle, hence on mysoin, is significant in view of the fact that sodium is known to enter muscle on stimulation. Excitation may result in increase of permeability, allowing sodium to enter and act on myosin.

Summary and Conclusions

(1) The similarity of the effects of ions on the protoplasmic viscosity of simple organisms and that on unstriated muscle suggest that the latter consists of a viscous protoplasmic non-contractile element. The similarity of the reactions of isolated myosin strips and isolated muscle suggests that it contains a contractile element. This view is supported by (*a*) the differential action of drugs, (*b*) viscous elastic properties of plain muscle, and by its histological picture.

(2) Similarity of the responses of isolated muscle and isolated myosin suggests that plain muscle can contract without the use of oxygen.

REFERENCES

Astbury, W. T., and Dicksonin, S. *Proc. Roy. Soc.*, B, 1940, **129**, 307.
Barth .. *Protoplasma*, 1929, **7**, 505.
Bouchaert, J. P., Capellen, L., and Blende, J. de *J. Physiol.*, 1930, **69**, 473.
Fletcher, C. M., .. *Ibid.*, 1937, **90**, 233.
Gokhale, S. K., and Singh, I. .. *Ibid.*, in the Press.
Heilbrunn, L. V. .. *Outline of General Physiology*, 1937.
———— .. *Amer. Jour. Physiol.*, 1923, **64**, 481.
——— and Daugherty .. *Physiol. Zool.*, 1931, **4**, 635.
———— .. *Ibid.*, 1932, **5**, 254.
———— .. *Jour. Cell and Comp. Physiol.*, 1934, **5**, 207.
Hill, A. V. .. *Proc. Roy. Soc.*, B, 1936.
Rao, M. S., and Singh, I. .. *J. Physiol.*, 1940, **98**, 12.
Roskin, G. .. *Z. F. Zellforschung*, 1925, **11**, 768.
Singh, I. .. *J. Physiol.*, 1936, **89**, 10.
———— .. *Ibid.*, 1937, **89**, 8.
———— .. *Ibid.*, 1938 *a*, **91**, 398.
———— .. *Ibid.*, 1938 *b*, **92**, 62.
———— .. *Ibid.*, 1938 *c*, **92**, 232.
———— .. *Ibid.*, 1938 *d*, **92**, 241.
———— .. *Ibid.*, 7938 *e*, **94**, 1.
———— .. *Ibid.*, 1938 *f*, **94**, 322.
———— .. *Ibid.*, 1939 *a*, **96**, 1.
———— .. *Ibid.*, 1939 *b*, **96**, 367.
———— .. *Ibid.*, 1940, **98**, 155.
———— .. *Ind. Journ. Med. Res.*, 1942 *a*, **30**, 449.
———— .. *Ibid.*, 1942 *b*, **36**, 29.
———— .. *Proc. Ind. Acad. Sci.*, 1943 *a*, **17**, 13.
———— .. *Ibid.*, 1943 *b*, **17**, 20.
———— .. *Curr. Sci.*, 1943 *c*, **17**, 13.
———— .. *Ibid.*, 1943 *d*, **12**, 56.
———— .. *Ibid.*, 1934 *e*, **12**, 83.
———— .. *Proc. Ind. Acad. Sci.*, 1943 *f*, **17**, 143.
———— .. *Ibid.*, 1943 *g*, **17**, 149.
Weber .. *Oesterreich. bot. Zeitschr.*, 1924, **73**, 261.
Winton, F. R. .. *J. Physiol.*, 1930, **69**, 393.

THE ELECTRICAL RESISTANCE OF UNSTRIATED MUSCLE AND OTHER TISSUES AND ITS RELATION TO PERMEABILITY AND EXCITABILITY

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THE study of the permeability of muscle is of great importance as it has exercised a great influence on the theories of excitation (for literature, see Heilbrunn, 1937); according to these theories increase in excitability is associated with increase in the permeability. The study of permeability is also of great importance from the standpoint of the theories of electrical excitation (Nernst, 1889; Hill, 1910), according to which the process of excitation by alternating current is essentially connected with production at some membrane of a certain minimal concentration of ions to whch the membrane is impermeable. Most of the phenomena produced by electric current can be produced by sudden increase in osmotic pressure of saline (Singh, 1942 *b*); this increases the concentration of the ions within the fibres (Singh, 1938 *a*; Gokhale and Singh). This suggests that electric current produces its effect by increasing the concentration of ions within the muscle fibres (Singh, 1936, 1937, 1938 *a, b, c, d, e, f*; 1939 *a, b*; 1940: 1942 *a, b*; 1943 *a, b*; *c, d, e, f, g, h*; Rao and Singh, 1940). An increase in permeability should therefore decrease the excitability to electric current, as thereby the ions would leak through the membrane and their effective concentration diminished. In *Mytilus* muscle the excitability to electric current is diminished in the absence of calcium, which is known to decrease the permeability of membrane. Agencies that increase the rate of leakage of potassium from the muscle fibres, such as lack of calcium, replacement of the chloride of the saline with thiocyanate decrease the excitability to electric current (Gokhale and Singh).

Decrease in the membrane resistance of muscle with excitation has been found by Duboisson (1934); Ebbeck, (1932); MeClendon (1912, 1927, 1936), Ray and Rapport (1927), Buchthall (1934); and increase has been found by Hogben and Gordon (1930), Achelis (1932), Bozzler (1935) and Bozzler and Cole (1935). Hartree found no change. Duboisson (1937) found an increase if the muscle was prevented from shortening.

Gelhorn and Gelhorn (1928) found that small doses of adrenaline decrease the permeability of frog muscle and large doses increase it. They also found that pilocarpine in doses 1 in 10,000 to 1 in 50,000 increased the

permeability and atropine, 1 in 10,000 to 1 in 30,000 decreased it. Osterhout (1912) found that ether of 1 p.c. increased and 3 p.c. decreased the resistance of *laminaria*; the resistance rose before decline.

Experimental

The muscles used were the smooth muscle of the frog stomach and also those of the abdominal wall of the frog *Rana Tigrina*; these muscles are suitable as they are flat. The electric resistance of the muscle was measured by simple apparatus ordinarily used in measuring the conductivity of solutions. It consisted of an audio-oscillator, head phones and a wheatstone bridge. The method was sensitive to 1 ohm with solutions, but with the muscle the sensitivity was greatly reduced ($\pm$ 20 ohms) and decreased with increase of resistance. The frequency used was 1,000 per sec.; other frequencies (600–800) made no appreciable difference.

The above method was successful, because a comparatively non-conducting solution was used; this consisted of a sucrose solution, the osmotic pressure of which was half that of normal and containing potassium chloride in strength 0·000112 M. The resistance of this solution was 8 times that of the muscle. As the muscle was inexcitable, errors due to contraction did not occur. Two kinds of muscle chambers were used. In the first kind the muscle was compressed between two circular discs of brass or copper, one inch in diameter and $\frac{1}{4}$ inch thick. The lower electorde was embedded in paraffin up to the upper surface in the base of a circular glass chamber; the electrode and the muscle remained immersed in the solution. The muscle was soaked in the various solutions, and then placed between the electrodes. In the second kind of chamber, the muscle was screwed between the two fixed metal electrodes, and immersed in a beaker containing the solution. This method was used to test the effect of electrolytes and has the advantage that the muscle is untouched during the course of the experiment. The muscle was also placed in a watch-glass and pressed by two platinum wires; drops of various substances were then added.

The effect of temperature was determined by the first method slightly modified. Circular electrodes were coated with shellac on all except the apposite surfaces, and were immersed in a beaker together with the muscle. The area of the muscle must be larger than that of each of the electrodes. In this way only about 2·5 p.c. of the current flows through the inter-fibre spaces.

The above methods are not so sensitive as used by other workers, but as only comparative values and variations of at least 100 ohms have been recorded, the results may be considered reliable; they are of same order of magnitude as found by more accurate methods (Hemingway and Collins, 1932).

Results

No significant difference has been noted between the reactions of striated and unstriated muscle.

Effect of the solution.—In half tonic sucrose solution (0·112 M $C_6H_{22}O_{11}$), the muscle keeps its resistance better than in solution of normal osmotic pressure. In the latter solution, the effects resulting in increase of resistance are not obtained; those resulting in a decrease are obtained. In full tonic solution the resistance tends to be low.

In a pure sucrose solution it is difficult to arrive at a steady state, which takes 3–4 hours; so electrolyte is added in very small concentrations. The best electrolyte was found to be potassium chloride (0·000112 M KCl). In this solution, the resistance of the muscles (1–2 mm. thick) was 500 ohms, or a specific resistance of 6,250 ohms. If sodium chloride is used, the resistance is about 400 ohms; if calcium chloride is used it is about 500–600 ohms. In pure sucrose solution it is about 500–800 ohms. In one season (January to April), these figures were remarkably constant in muscles from many different animals. In the hot weather (May), the resistance was lower, and the results difficult to obtain.

To obtain constant and positive results the condition of the muscle must be good; if the resistance falls below the figures quoted above, then the results are inconsistent.

When the muscle is immersed in sucrose electrolyte solution, the increase in the resistance reaches a constant value in 1½–2 hours and keeps so for several hours. The sucrose solution is in no way harmful to the muscle. After initial depression of excitability for a couple of hours the frog stomach shows spontaneous contractions and remains irritable to direct current for about 6 hours, and then passes into contracture with permanent loss of excitability. It is remarkable how the muscle remains irritable in the complete absence of sodium chloride; since there are hardly any ions outside, the excitation in this instance is probably produced by ions inside the muscle fibres.

If the muscle is immersed in the sucrose electrolyte solution of normal osmotic pressure, then the muscle becomes non-irritable. This is probably due to the fact, that when the muscle is immersed in such a solution, there is so much ionic unbalance within and without the muscle fibres, as to upset the excitatory mechanism. As the depressant action of ions on one side of the muscle membrane is counteracted by ions on the other side (Singh, 1939 *b*) the unantagonised depressant action of ions within becomes manifest, when ions are removed from without the muscle fibres. If the osmotic pressure of the saline is reduced, the concentration of the ions within and hence then

the depressant action is diminished. The muscle however recovers irritability when it is replaced in normal environment. In pure sucrose solution too, the muscle becomes non-irritable, but recovers when replaced in saline. In all these sucrose solutions, the frog skeletal muscle loses irritability in a few minutes, but recovers when re-immersed in Ringer Solution.

As the environment was abnormal, the experiments were concluded as quickly as possible. Observations were made every 15 minutes or half an hour. Three constant readings were first obtained. This occupied 2 hours if observations were made at 15 minutes-intervals, or $2\frac{1}{2}$ hours with half-hourly observations. The muscle was then immersed for 15–30 minutes in the experimental solution, and then again in the control solution the experiment being over in $2\frac{1}{2}$–4 hours.

The condition of the muscle is also shown by studying the permeability of the muscle to water; the muscle keeps in good condition for the period experimented upon.

Effect of death of muscle.—The muscle was killed by immersion for 10–15 seconds in solution heated to 70–80° C. and by immersion in absolute alcohol. The resistance of 12 striated and 2 unstriated muscles was 400 ohms each. After heating it was 100 ohms in each case. The resistance of 10 muscles in pure sucrose solution was 600, 600, 700, 500, 400, 500, 400, 500, 360, 500 ohms respectively; after death it was 100 ohms for each muscle. Measured by the watch-glass method the resistance to direct current of six muscles respectively was 20,000, 22,000, 22,000, 18,000, 19,000, 22,000 ohms in pure sucrose solution; after death it was 6,000–7,000 ohms. The resistance of 12 muscles respectively in pure sucrose solution was 1,100, 1,000, 700, 500, 600, 700, 600, 600, 800, 700, 800, 700 ohms; after immersion in absolute alcohol, it fell to 300, 300, 700, 300, 500, 300, 400, 600, 400, 300, 300, 300 ohms respectively.

As killing destroys the muscle membrane it is evident that the resistance of the muscle membrane is higher than the interior of the muscle, and the electrical resistance is a measure of the permeability of the muscle membrane.

Effect of passage of direct current.—When direct current is passed through the muscle, polarisation occurs, so that the value of the current rapidly falls, and if now the two terminals are switched over to a galvanometer, a current flows in the opposite direction. The polarisation occurs in the muscle, as it is little marked in the absence of the muscle.

Thus when the current is passed, a concentration of ions takes place in the muscle; this is probably the concentration postulated by the various theories of electrical excitation (Nernst, 1899; Hill, 1910).

The effect of the passage of the direct current is to reduce the resistance of the muscle. The resistance of 12 striated muscles was 400 ohms each. After passage of D.C. 1 volt for 30 sec. it was reduced to 300 ohms each; passage of 2 volts for 15 seconds, reduced it to 200 ohms each; this however produces visible electrolysis.

Effect of temperature.—The resistance is low at low (below 5° C.) and at high temperatures (above 40° C.). Between these temperatures it does not change very much; the temperature curve shows one or two maxima at 10° C. and between 25–30° C. (Table I) respectively, or the resistance may decline

TABLE I

Effect of temperature on resistance of tissues

A. Frog muscle

°C.	Solution	Dead muscle	No. of living muscle											
			1	2	3	4	5	6	7	8	9	10	11	12
2	5100	500	500	..	550	500	500	500	600	600	500	500	500	600
5	4500	500	600	500	600	600	600	600	700	700	700	600	600	600
10	4100	450	600	600	700	600	700	700	700	700	700	700	700	600
15	3700	400	600	500	600	500	600	500	500	500	600	600	600	500
20	3300	400	500	500	450	500	500	400	400	400	500	500	500	500
25	2900	400	600	800	450	500	500	500	600	600	500	500	500	500
30	2600	350	650	700	400	500	600	500	500	500	600	600	600	500
35	2400	300	500	500	350	400	500	400	400	400	500	500	500	400
40	2200	300	400	400	300	400	400	400	400	400	500	500	500	400
45	2000	300	300	300	300	300	300	300	300	300	400	300	300	300
50	1800	200	200	200	200	200	200	200	200	200	300	200	200	200

B. *Resistance of guinea pig muscle, frog gastric mucous membrane (m.m.), frog skin, and frog liver. Frog skin about $\frac{1}{4}$ thickness that of muscle; frog gastric mucous membrane about $\frac{1}{2}$*

°C.	Guinea pig muscle No.				Frog skin No.			Frog m.m. No.			Frog liver No.		
	1	2	3	4	1	2	3	1	2	3	1	2	3
2	500	600	600	600	800	900	800	600	600	600	900	1000	900
5	600	700	700	700	800	800	800	700	600	700	1000	1000	1000
10	600	700	600	600	700	700	700	700	700	700	1100	1000	1100
15	500	600	600	600	600	600	500	600	600	600	1000	1000	1000
20	400	500	500	500	500	500	500	500	600	500	800	800	800
25	500	500	500	500	500	500	400	600	600	400	800	800	800
30	400	500	400	400	400	400	400	300	500	400	900	800	900
35	400	400	400	400	400	400	400	400	400	400	700	700	700
40	400	400	400	400	300	300	300	400	400	400	700	700	700
45	300	300	300	300	300	300	300	300	300	300	600	600	600
50	200	200	200	200	300	250	250	200	200	200	500	500	500

continuously with temperature; the decline may be slow till 40° C., and more rapidly thereafter.

The resistance of frog muscle is permanently lowered if heated beyond 35° C., and that of mammalian muscle if heated beyond 40° C. If the resistance of dead and living muscles is compared, death being produced by heating at 50° C., the resistance of the two approximate below 5° C., and above 40° C. This is due to the fact that energy is required to maintain the normal permeability of the muscle. At low temperatures the diminution of chemical processes would account for the high permeability; this view is supported by the fact that asphyxia lowers the resistance. This is in agreement with fact, that muscles from mussels stored at low temperatures contain more sodium and base than normal muscles (Singh, 1938 *a*).

The maximum resistance between 25°–30° C. corresponds to maximum excitability to electric current and minimum tone of frog stomach. The minima at low and high temperatures and at 20° C. correspond to high tone. Increase in permeability, therefore, increases the sensitivity to ions without and decreases that to electric current. The increased excitability of tissues at low temperatures is due to increased permeability.

At temperatures below 5° C. and above 40° C., the excitability decreases, showing that an optimum permeability is necessary. If the permeability is too much increased then inexcitability results (Singh, 1943 *f*).

Continuous increase in permeability with temperature corresponds to continuous increase of tone.

The resistance of frog skin is much higher than that of muscle, that of the gastric mucous membrane having an intermediate value. This would correspond to the protective function of the skin and the mucous membrane.

At high temperatures potassium rapidly leaks from the muscle and raises the conductivity of the solution.

Sudden lowering of the temperature from 40° C. may result in a higher resistance than if the temperature is gradually lowered; this is probably due to liberation of calcium.

Effect of radiation.—This is complex and depends upon some unknown factors. The resistance of 25 muscles (temperature 27° C.) was 500 ohms. After 5 minutes exposure to sunlight, the resistance rose to 700 ohms in every one of them and the increase was completely reversible. The muscles were exposed to sunlight through a layer of the solution, which was changed continuously to eliminate the effect of heat, which however has an opposite action. 30 Minutes exposure produced an opposite action, the resistance being lowered to 400 ohms.

In another series of experiments on 12 muscles at 30° C., the resistance of each muscle was 400 ohms. After 5 minutes exposure to sunlight the resistance was 500, 500, 500, 500, 500, 500, 500, 400, 400, 400, 400, 400 ohms respectively; the effect was complete reversible. The same effect was produced if a blue filter was interposed, 30 minutes exposure produced a decrease.

In a third series of experiments on 18 muscles at 32° C., the resistance of each muscle was 400 ohms. Exposure to sunlight or blue light reduced the resistance to 300 ohms; no increase was recorded.

In a fourth series of experiments on 36 muscles at 34° C., the resistance of each muscle was 300 ohms. Exposure to sunlight or blue light produced no effect.

In some muscles in which the resistance decreased, the return to normal was preceded by a stage of increased resistance. Radiation therefore effects two opposing factors; the increase is probably due to liberation of calcium. These results are in agreement with the observation that radiation may increase the excitability of plain muscle (Guttman and Wilbur, 1936) or decrease it (Harris, 1925). Radiation from isotopes decreases the permeability to sodium (Blinks, 1942).

Mechanical effects.—Blotting of the muscle preliminary to weighing, decreased the resistance of 12 muscle from 400 ohms to 300 ohms each. This is in agreement with the fact that *Mytilus* muscle gains more base and weight, if blotted (Singh, 1938 *a*). This is also in agreement with the fact that plain muscle may contract if touched or stroked on the surface. Mechanical stroking thus increases the permeability and tone of the muscle.

Effect of asphyxia.—The resistance of 6 muscles was 600 ohms each. Deprival of oxygen lowered the resistance to 300–400 ohms; the same figure was obtained if the muscle was killed with absolute alcohol. Asphyxia thus increases the permeability of the muscle, and if the condition of the latter is good, this results in increased excitability. Increase in tonus of plain muscle is known as a result of oxygen lack. A decrease in the rate of relaxation also occurs, indicating increased excitability to ions outside and hence increased permeability.

Effect of cations.—In 25 muscles replacement of NaCl with KCl increased the resistance from 400 to 500 ohms. Out of 12 muscles, $CaCl_2$ increased the resistance from 400 to 500 ohms in 9 and to 600 ohms in 3 muscles. $MgCl_2$ increased the resistance from 400 to 500 ohms in 6 muscles.

The above cations in small concentrations produce inhibition in unstriated muscle; so it appears that excitation is associated with increased permeability,

and inhibition with an opposite change; the excitability to electric current is, however, oppositely affected. These results are in agreement with the fact that in *Mytilus* muscle, (1) calcium lack increases the gain of sodium, potassium and barium (Singh, 1938 *a*) and leakage of potassium (Gokhale and Singh), (2) small concentrations of potassium (0·01 M KCl) decreases the gain of sodium, barium and strontium, (3) small concentrations of magnesium diminish the gain of sodium. Calcium and magnesium are known to decrease the permeability of cells.

The effect of large concentrations of these ions could not be tested, but if the muscle was soaked in 0·154 M KCl for 1½ hours, it completely recovered if replaced in sucrose solution. Large concentrations such as 5 p.c. KCl damaged the muscle. Similarly recovery was affected from 0·5 M NaCl, the concentration in *Mytilus* saline. Sodium and potassium are known to increase the permeability. Ammonium acted like potassium.

Effect of anions.—The following procedure was adopted to test the effect of electrolytes. The muscle was soaked in pure sucrose solution. Using the second apparatus described, a small concentration of electrolyte such as NaCl was added. There was immediately a sharp fall in resistance. After a few minutes, the pure solution was added. If the resistance passed through a supernormal phase before it returned to normal, then small concentration of the electrolyte increased the resistance, because, when the muscle is replaced in the pure sucrose solution, then the electrolyte must diffuse out and its concentration immediately around the muscle must diminish. In this way it was found that sodium chloride and other salts mentioned herein increased the resistance.

An increase of resistance by electrolytes is to be expected, as the normal permeability is maintained by ions in the environment. The anions increased the resistance in the order $Cl = Br = NO_3 \leqslant 1 \leqslant SCN$. The cyanide ion in small concentrations also increased the resistance.

The above ions in small concentrations, cause inhibition of tone in plain muscle in the order $Cl = Br \leqslant NO_3 \leqslant 1 \leqslant SCN$, so that inhibition is associated with decreased permeability. Large concentrations of SCN (0·5M) decrease the resistance, the method used being as with potassium. In *Mytilus* muscle large concentrations of these ions increase the gain in sodium and the excitability to ions without in the above order, the excitability to electric current being affected in the opposite direction. In frog muscle, the loss of potassium is also affected in the same order. Increased permeability of the muscle membrane thus increases the excitability to ions without and decreases that to electric current.

Effect of adrenaline.—The effect of this is most striking. In 40 muscles, small concentrations (1 in 10^8 to 10^7) at 27° increased, and 1 in 10^6 decreased the resistance (Table II). When the muscle is restored to the original solution from adrenaline 1 in 10^6, a supernormal stage is observed, as smaller

TABLE II

Effect of adrenaline on resistance (Ohms)

Solution	No. of muscle										
	1	2	3	4	5	6	7	8	9	10	11
Normal	400	400	400	400	400	400	400	400	400	400	400
„	400	400	400	400	400	400	400	400	400	400	400
„	400	400	400	400	400	400	400	400	400	400	400
1 in 10^6 adrenaline	400	400	300	400	400	400	400	400	400	400	400
Normal	800	800	800	800	800	800	800	800	800	800	800
„	500	600	700	500	500	500	500	500	500	500	500
„	500	500	500	500	500	500	500	500	500	500	500
1 in 10^6 adrenaline	700	700	800	1000	700	800	700	800	700	800	1000
Normal	500	500	500	600	500	500	500	500	500	500	800

concentrations increase the resistance. The second method gives similar results (Fig. 1); besides it shows that it is a surface effect.

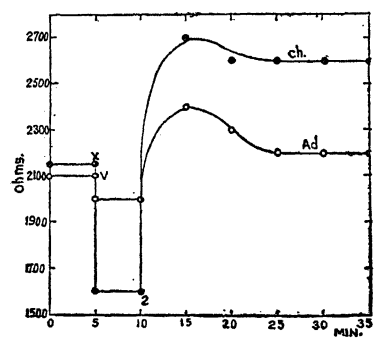

Fig. 1. Effect of adrenaline (Ad) and chloral hydrate (CH) on resistance
1 p.c. chloral hydrate added at *x* and withdrawn at Z.
1 in 10^7 adrenaline added at V, and withdrawn at Z.

At higher temperature (33 °C.), when the resistance declines, the increase in resistance with adrenaline is not obtained (20 muscles). In sucrose solution of normal osmotic pressure, which also reduces the resistance, adrenaline is without effect (12 experiments).

In *Mytilus* muscle small concentrations of adrenaline cause inhibition, and larger concentrations, contraction; inhibition is thus associated with decreased, and excitation with increased permeability. The excitability to alternating current is affected oppositely. In frog muscle 1 in 10^7–10^8 adrenaline

increases the excitability to electric current, and decreases that to potassium. In 10^6 adrenaline decreases the excitability to both, so that it appears that for excitability an optimum permeability is necessary; decrease or too much increase in permeability renders the muscle less excitable.

Effect of chloral hydrate.—This has a powerful effect on the muscle. In 20 muscles small concentrations (1 in 1,000) increased and large concentrations decreased the resistance (Table III). The second method gives similar results

TABLE III

Effect of chloral hydrate on resistance

Solution	No. of muscle									
	1	2	3	4	5	6	7	8	9	10
Normal	450	450	450	450	450	440	430	450	450	450
,, ...	440	450	440	450	450	450	440	450	450	450
,, ...	450	450	450	450	450	450	450	450	450	440
1 in 10^3 chloral hydrate	1000	800	700	1000	750	650	650	750	450	700
1 in 100	600	600	700	500	400	500	400	500	300	500
Killed by heat	100	100	100	100	100	100	100	100	100	100

(Fig. 1) with sucrose solution of normal osmotic pressure, increase in resistance is not obtained; 1 in 1,000 chloral hydrate only causing a decrease (12 experiments). Chloral hydrate increases the conductivity so it cannot be used in high concentrations.

Effect of chloroform.—Small concentrations increase and large concentration decrease the resistance. The resistance of 10 muscles respectively was 400, 500, 500, 500, 500, 400, 400, 400, 500, 500 ohms. 1 in 1,000 chloroform changed it respectively to 500, 700, 600, 500, 600, 400, 400, 500, 500, 500 ohms. 1 in 250 changed it to 500, 700, 600, 700, 700, 800, 700, 600, 700, 600 ohms.

Effect of ether.—Small concentrations increase and large concentrations decrease the resistance. The resistance of 10 muscles respectively was 600, 600, 600, 700, 700, 600, 600, 600, 600, 600 ohms. Ether 1 in 1,000 changed it to 600, 900, 1,100, 700, 700, 200, 900, 900, 700, 800 ohms; 1 in 100 changed it to 500, 600, 300, 600, 400, 400, 500, 400, 400, 500 ohms. This concentration of ether increases the excitability to potassium and decreases that to A.C. in *Mytilus* muscle.

Effect of ethyl alcohol.—Small concentrations increase and large concentrations decrease the resistance. The resistance of 12 muscles respectively was 500, 700, 700, 500, 600, 600, 600, 600, 600, 700, 500, 500 ohms. 1 in 1,000 alcohol changed it to 500, 700, 700, 500, 700, 600, 600, 600, 700, 500, 500, 500 ohms. 1 in 100 changed it to 600, 700, 700, 700, 700, 600, 600, 700, 600, 600, 600,

600 ohms. 1 in 10 changed it to 1,100, 1,000, 700, 500, 600, 700, 600, 800, 700, 800, 700, 700 ohms. 1 in 1 changed it to 300, 300, 700, 300, 500, 300, 400, 600, 400, 700, 300, 300 ohms.

Effect of butyl alcohol.—As this evaporates quickly the watch-glass method was used, the muscle being pressed between 2 electrode wires. The watch-glass contained 6 c.c. of solution, and 3 or 4 drops of butyl alcohol added. In 10 minutes the resistance of three muscles rose from 2,600, 2,600, 2,500 ohms respectively to 3,000, 3,100, 3,000 ohms.

Effect of octyl alcohol.—The resistance of 12 muscles respectively was 500, 500, 400, 400, 400, 500, 500, 500, 500, 600, 500 ohms. 1 in 1,000 octyl alcohol reduced it to 300 ohms each; this is the best substance for reducing the resistance.

Effect of novocaine.—The resistance of 12 muscles respectively was 500, 500, 500, 600, 600, 600, 600, 600, 600, 500, 500, 500 ohms. 1 in 10^6 novocaine changed it to 600, 600, 700, 800, 900, 700, 1,000, 1,200, 1,000, 800, 800, 800 ohms.

Effect of caffeine.—The resistance of 12 muscles respectively was 500, 500, 500, 600, 500, 500, 500, 500, 500, 800, 500, 500 ohms. 1 in 10^6 caffeine changed it to 700, 700, 800, 900, 700, 700, 800, 600, 900, 800, 800, 800 ohms. In plain muscle small concentrations of caffeine increases the excitability to electric current.

Mammalian muscle and nerve.—The effect of temperature resembles that on frog muscle, but effects resulting in increase of resistance and other effects are not obtained; the sucrose solution is presumably not conducive to these changes. Liver is also not affected (animal used, guinea pig).

Discussion

The changes in the resistance of the muscle to alternating current of low frequency does appear to represent changes in permeability as shown by the close correspondence between the results of some diffusion experiments and changes of resistance. As the muscle membrane is a part of the cell, it is bound to be affected by agencies of biological significance, but the question is whether the changes so produced affect the activity of the cell as described above. It appears that inhibition is associated with decrease of permeability and excitation, with an opposite change. For excitation an optimum permeability appears necessary, the excitability decreasing if the permeability is decreased or increased beyond a limit.

The relation between permeability and excitability of smooth muscle is also shown by diffusion experiments. *Mytilus* muscle gains base when the

sodium or the chloride of the saline is replaced by other cations or anions respectively in the order.

$$Li < Na < NH_4 < K$$
$$Cl < Br < NO_3 < I < SCN < CN$$
$$Ca < Sr < Ba$$

The stimulating power of the cations and the anions varies in the same orders, and the anions increase the excitability to potassium in the same order. The excitability to alternating current is oppositely affected. It thus appears that increase in permeability increases the excitability to chemical stimulation.

The doctrine of permeabilty, advocated chiefly by Hober in Germany and Lillie in the United States, has had support at one time or other of many distinguished physiologists, among others, Winterstein, Osterhout, McClendon, Jacobs, Gildmester, Ebbecke, Gelhorne. Rosenbleuth and Cannon (1936) explain the increased sensitivity to adrenaline brought about by cocaine as due to increase in permeability. Recent experiments of Cole also show a decrease of resistance following excitation. In *Mytilus* muscle cocaine and veratrine *increase* the excitability to potassium, and these reagents decrease the resistance of nerve (Gerard, 1942).

There is a striking resemblance between the curve showing sensitivity of *Mytilus* muscle to ions (Singh, 1938*f*) and the impedance changes described by Duboisson. The curve shows an increase or decrease of sensitivity preceded by an increase; Duboisson found a rapid decrease of impedance followed by an increase or decrease of impedance.

The fact that the muscle membrane is affected by two distinct agencies, such as ether, chloroform and alcohols on the one hand, and salts and drugs on the other, support the idea that it is a lipid protein complex. The fact that the ions which affect the permeability (as shown by diffusion) can be arranged in the order of the lyotropic series, also point to its colloidal constitution. The fact that plain muscle is more permeable to thiocyanate ion than to the chloride ion (as shown by the gain of base by *Mytilus* muscles, and loss of potassium by frog stomach) shows that permeability is dependent upon factors other than pores. The fact that the muscle is more permeable to ammonium and potassium than sodium or lithium, however, suggests a pore factor.

The existence of two factors in the permeability of smooth muscle is shown by the effects of veratrine and thiocyanate. Both these substances increase the excitability to potassium; with the latter, the muscle gains base, but veratrine has no significant effect on the base content.

Now that rectification has been definitely established for the nerve membrane, some phenomena of smooth muscle can also be explained, on a similar assumption. Some effects of direct current can be produced by alternating current of greater intensity. Thus if *Mytilus* muscle is sensitive, the delayed relaxation produced by direct current of 8 volts can be produced by alternating current of 16 volts or more. If both alternating and direct currents produce a concentration of ions in some part of the muscle, only one direction of the current appears to be effective, so that to produce the effect of direct current, alternating current of greater intensity has to be used.

Rectification is also suggested by chemical analyses. In alkaline solutions (pH 8) *Mytilus* muscle gains more base than in acid solutions; the permeability of erythrocytes is similarly affected (Blinks, 1942). But, though with erythrocytes the membrane becomes porous both ways, with smooth muscle the opposite happens. Potassium leaks more readily in acid than in alkaline solutions (Gokhale and Singh), as also found in skeletal muscle (Fenn and Cobb, 1935).

Summary and Conclusions

(1) As shown by the effect of death, resistance of the muscle to alternating current of low frequency is a measure of the permeability of the cell.

(2) Permeability is high at low and high temperatures; frog muscle if heated beyond 35° C. and mammalian muscle beyond 40° C., is irreparably damaged, the resistance being permanently lowered.

(3) Cations such as potassium, calcium, ammonium, magnesium increase the resistance of the muscle in small concentrations.

(4) Anions such as Cl, Br, I, NO_3, SCN, CN increase the resistance of the muscle in small concentrations.

(5) Drugs and narcotics such as adrenaline, caffeine, novocaine, chloral hydrate, ether, chloroform, ethyl alcohol, butyl alcohol, octyl alcohol increase the resistance in small and decrease it in large concentrations.

(6) Inhibition and narcosis are associated with increased resistance or decreased permeability. Increased excitability to ions without is associated with an opposite change. Excitability to electric current is increased with diminished permeability.

(7) For excitation an optimum permeability is necessary.

(8) The resistance of frog skin is higher than that of muscle.

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REFERENCES

Achelis, J. D. .. *Pfluger's Arch.*, 1932, 320, 412.
Blinks, L. R. .. *Annual Rev. Physiol.*, 1942, **1**, 4.
Bozzler, E. J. Cell .. *Comp. Physiol.*, 1935, **6**, 217.
Bozzler, E. .. *J. Cell. Comp. Physiol.*, 1935, **6**, 217.
——— and Cole, S. .. *Ibid.*, 1935, **6**, 229.
Buchthal, F. .. *Skand. Arch. Physiol.*, 1934, **68**, 187.
Duboisson, M. .. *Arch. int. Physiol.*, 1934, **38**, 85.
——— .. *J. Physiol.*, 1937, **89**, 132.
Ebbecke, U. .. *Pfluger's Arch.*, 1922, 195, 555.
Fenn, W. O., and Cobb, D. .. *Amer. J. Physiol.*, 1936, 115, 345.
Gelhorn, E., and Gelhorn, H. .. *Pfluger's Arch.*, 1928, 221, 247.
Gerard, R. W. .. *Annual Rev. Physiol.*, 1942, **4**, 329.
Gokhale, S. K., and Singh, I. .. *J. Physiol.*, in the Press.
Guttman, S. A., and Wilbur, D. T. *Amer. Jour. Physiol.*, 1936, 194, 115.
Heilbrunn, L. V. .. *Outline of General Physiology*, London.
Harris, D. T. .. *Proc. Roy. Soc.*, B, 1925, 399,110.
Hemingway, A., and Collins, D. A. *Amer. J. Physiol.*, 1932, **99**, 338.
Hill, A. V. .. *J. Physiol.*, 1910, **40**, 190.
Hogben, L., and Gordon, E. .. *J. Expt. Biol.*, 1930, **7**, 269.
McClendon, J. F. .. *Amer. J. Physiol.*, 1912, **29**, 302.
——— .. *Ibid.*, 1927, **82**, 585.
——— .. *Science*, 1936, **84**, 184.
Nernst, W. .. *Golt. Nachr. Math. Phys. Kl.*, 1899. Quoted from Hill, A. V., *J. Physiol.*, 1910, **40**, 190.
Osterhout, W. J. V. .. *Science*, 1912, **37**, 111.
Ray, G. B., and Rapport, D., .. *Amer. J. Physiol.*, 1927, 80, 126.
Rao, M. S., and Singh, I. .. *J. Physiol.*, 1940, 98, 12.
Rosenbleuth, A., and Cannon, W. B. *Amer. J. Physiol.*, 1936, 414, 116.
Singh, I. .. *J. Physiol.*, 1936, **89**, 10.
——— .. *Ibid.*, 1937, **89**, 8.
——— .. *Ibid.*, 1938 *a*, **91**, 398.
——— .. *Ibid.*, 1938 *b*, **92**, 62.
——— .. *Ibid.*, 1938 *c*, **92**, 232.
——— .. *Ibid.*, 1938 *d*, **92**, 241.
——— .. *Ibid.*, 1938 *e*, **94**, 1.
——— .. *Ibid.*, 1938 *f*, **94**, 322.
——— .. *Ibid.*, 1939 *a*, **96**, 1.
——— .. *Ibid.*, 1939 *b*, **96**, 367.
——— .. *Ibid.*, 1940, **98**, 155.
——— .. *Ibid.*, 1940, **98**, 155.
——— .. *Ind. Journ. Med. Res.*, 1942 *a*, **30**, 449.
——— .. *Ibid.*, 1942 *b*, **36**, 29.
——— .. *Proc. Ind. Acad. Sci.*, 1943 *a*, **17**, 13.
——— .. *Ibid.*, 1943 *b*, **17**, 20.
——— .. *Curr. Sci.*, 1943 *c*, **12**, 56.
——— .. *Ibid.*, 1943 *d*, **12**, 57.
——— .. *Ibid.*, 1943 *e*, **12**, 83.
——— .. *Proc. Ind. Acad. Sci.*, 1943 *f*, **17**, 143.
——— .. *Ibid.*, 1943 *g*, **17**, 149.
——— .. *Ibid.*, 1943 *h*, **18**, 53.

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A CRITICAL REVIEW OF SOME RECENTLY CREATED NEW SPECIES OF INDIAN ZYGNEMALES

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(*Rae Bareli*)

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THE members of the order Zygnemales have attracted a good deal of attention recently in U.S.A., Europe, China and India. A number of new species and two new genera have been described. Of the work done outside India, the investigations of Transeau and his colleagues Tiffany, Taft and Li deserve particular mention. Transeau has described a number of new forms and has also given a systematic account of the species belonging to different genera in a series of monographs, which have proved exceedingly useful to workers in this line. Recently he brought out a mimeographed key of all the known species, which is invaluable to all those who are interested in the systematic study of this group. Jao and Li have published valuable monographs on the hitherto little known members of this group from China.

In Europe valuable work has been done by Czurda and Skuja on members of this order. In spite of differences of opinion one may have regarding the delimitation of various genera, Czurda's keys given in Heft 9 of *Die Susswasserflora Mitteleuropas* are of considerable use to the workers. His recent monograph on the "Morphology of Conjugatæ" is an excellent treatise on this group and is very well illustrated.

In India pioneer work on Zygnemales was done by Iyengar whose short note on "Studies on Zygnemales" with detailed observations on peculiarities in conjugation is valuable.

The present author published a series of papers on this group and recently described an interesting new genus, which he has named Sirocladium, from Kumaon Himalayas. Some work has also been recently published on this group by Rao, Dixit, Misra and Singh from the Benares Hindu University under the direction of Prof. Bharadwaja, describing a number of new species.

As a number of young workers have interested themselves in this group it seems desirable to review some of the species described as new by the various authors. In the systematic studies of this group, the sculpturing of the spore-wall is of fundamental importance particularly in species of *Zygnema, Spirogyra*

and *Sirogonium*. A difference of a few microns in the width of vegetative cells or in the dimensions of zygospores is quite immaterial in most cases as it may be due to physiological conditions. In fact even in the same species there may be a wide range in the size of vegetative cells or zygospores. Only when the alga shows some marked peculiarity in conjugation as in *Zygogonium taluguppense* Iyengar and *Zygnema Czurdæ* Randhawa, that a valid species can be based even if the zygospores are immature, irrespective of the sculpturing of the spore-wall.

Some of the species described as having smooth spore-wall, have been later on found to possess minute punctation. The species which were described in earlier days when microscopic technique was not so well advanced are in need of revision and re-examination. Sculpturing of the mesospore can only be observed at a magnification of × 800 or more and the use of an oil immersion lens is imperative. There is another important detail which a worker should always keep in view. When observing the sculpturing of the spore-wall, *open the diaphragm of the condenser shutter fully*. When the diaphragm is closed, in most instances only reticulations are seen in the spore-wall. The present author made a similar mistake when describing the sculpturing of the spore-wall of *Zygnemopsis lamellata* Randh. which was originally described as reticulate, and later on it was found to be punctate. Another helpful tip is the use of 30% chromic acid to dissolve the cell contents, or by lightly crushing the spores under the cover-slip. This is particularly helpful when the spore-wall is finely punctate.

There is another mistake which one is likely to make when describing species, in which the dimensions and the shape of the vegetative cells is so alike that differences are only seen when mature spores appear. In algæ it is so rare to find pure material, for species are so much mixed in nature, and this is likely to create confusion when the vegetative cells are more or less alike in dimensions. This type of mistake is more likely to arise in the case of the species of *Zygnemopsis* in which aplanosporic species and zygosporic species are usually mixed. In his original description of *Zygnemopsis lamellata* the present author described both zygospores and aplanospores, which are usually found together. Later on more minute examination of the spore-wall revealed that the aplanosporic form is a different species with a pitted spore-wall in the aplanospores, while the zygospores which belong to the species *Z. lamellata* are punctate.

Sculpturing and pigmentation develop in the mesospore of the spore on maturity. Immature spores have thin walls and chloroplasts are clearly visible. Such material is usually of no value for investigation purposes and

cannot safely form the basis of a new species unless there are some marked peculiarities in the mode of their reproduction. Mature spores are usually deep blue, orange, or chocolate coloured, are always opaque and never show chloroplasts. When material with ripe spores is collected, the sculpturing of the mesospore should be carefully observed. The spore-wall is very rarely smooth in the species of *Zygnema* though there are some species of *Spirogyra* and *Mougeotia* in which it is smooth. Usually the following types of sculpturing has been observed in species described from India, and in some cases there may be combination of two or more types :—(1) Punctate, (2) Scrobiculate or pitted, (3) Reticulate, (4) Denticulate, and (5) Verrucose.

When the spore-wall is pitted the size of pits, and their distance from each other should be carefully measured.

In the identification of species of *Spirogyra* the type of septa should be carefully observed, to find out whether it is plane, replicate, semi-replicate or colligate. In species of *Spirogyra* and *Sirogonium* where the number of chloroplasts exceeds three, it becomes very variable, and in itself is a very unsafe character to base a new species. In a sample of *Sirogoium ventersicum* Transeau, the present author found 6–9 chloroplasts. In a similar material which Dixit described as *S. inflata* sp. nov. (1937) he found 10 chloroplasts. Later on a similar material was described by Singh (1938) as *S. indicum* sp. nov. and the number of chloroplasts is given as 7. The present author has found that in *Sirogonium ventersicum*, the number of chloroplasts varies from *4–10* and to pick up any individual cell and to say that it contains 7 or 10 chloroplasts and then to base a new species on it is rather a risky venture. Similarly the number of turns of a chloroplast is also variable and is an unreliable guide in species of *Spirogyra*.

The shape of zygospores is also variable in species of *Spirogyra* and globose to ellipsoid zygospores may be found in the same species or in some cases in the same filament. Similarly when the fruiting cells are inflated, the extent of inflation is very variable and is not of much significance. The size of zygospores is also variable and by itself is not a safe criterion for making a new species.

The following is a critical account of some doubtful species which have been described as new and a re-investigation seems to be necessary to clear the doubts.

Zygnema Agardh

1. *Zygnema gangeticum* Rao.—The author has described the mesospore as *thin, smooth* and yellowish-brown. This feature as well as his figures show that he did not come across mature zygospores. The so-called *thinness*

of the mesospore is due to its immaturity. A similar form was described by the present author as Z. *heydrichii* Schmidle var. *indicum*. The present author is of the opinion that *Z. gangeticum* Rao *is not a valid species*, and is merely a variety of *Z. heydrichii* Schmidle.

2–4. The three species: 2. *Z. indica* Misra: 3. *Z. sphærica* Misra and 4. *Z. Kashmiriensis* Misra—were described by Misra as new and are based on studies of material collected by Bharadwaja from Srinagar, Kashmir. In the case of *Z. indica*, the mesospore is described as " slightly thick, smooth, and blue ". In the case of *Z. Kashmiriensis*, the mesospore is described as " thin, smooth and brown ". However, the figures of the author clearly show that he was sketching immature zygospores which show chloroplasts. In *Z. sphærica* he described the mesospore as " *thin, smooth* and blue ". This description shows that he came across immature zygospores only. The present author is of the opinion that all the above-mentioned three species are invalid being based on the study of immature zygospores. Moreover, too much reliance was placed on the keys of Czurda, which have become out of date, as a number of new species have been described since then from U.S.A., China and India.

Spirogyra Link.

1. *S. Czurda* Misra.—According to Misra " the presence of a scrobiculated mesospore is a unique feature of this alga ". However, a scrobiculate mesospore is found in a few known species of *Spirogyra*, such as *S. luteospora* Czurda. Comparison of Misra's description of *S. Czurda* with that of Transeau of *S. luteospora* Czurda given in his " mimeographed key " shows that both these algæ have a single chloroplast, vegetative cells are of the same size, dimensions of zygospores are similar and spore-wall is scrobiculate in both. This leads one to the conclusion that *S. Czurda* Misra is not a valid species and Misra has actually recorded *S. luteospora* Czurda.

2. *S. Skuja* Randhawa and 3. *S. reticuliana* Randhawa were described by the present author as new. Further observations on the material of these algæ shows that they differ from each other only in minor features. The dimensions of the vegetative cells and zygospores are more or less similar. The description of *S. reticuliana* covers that of *S. Skuja* and the latter does not deserve to be treated as a separate species.

4. *S. paradoxa* Rao.—According to Rao this alga differs from *S. setiformis* (Roth) Kutz. " in the possession of narrower cells, lesser number of chloroplasts, and the fructifying cells which are commonly swollen on the conjugating side ". Narrower cells may be due to poor nourishment, and the number of choloroplasts when it exceeds three is a very uncertain feature.

The swelling of fructifying cells towards inside is not an important feature specially when it is not constant. The present author is of the opinion that *S. paradoxa* Rao is merely an attenuated form of *S. setiformis* (Roth) Kutz.

5. *S. azygospora* Singh.—While Singh has described the mode of reproduction as by means of *azygospores*, he actually gives the length and breadth of *zygospores*. This is possibly a mistake. Formation of *azygospores* is, however, not a permanent feature in any member of the Zygnemales, as due to interruption of normal conjugation azygospores may be formed in any species which normally produces zygospores. The production of azygospores is only a physiological feature and is not of any morphological and systematic importance. In dimensions of cells and number of chloroplasts Singh's *S. azygospora* resembles *S. submaxima* Transeau. In the opinion of the present author *S. azygospora* Singh is merely the azygosporic form of *S. submaxima* Transeau and therefore need not be treated as a separate species.

Sirogonium Kutz.

1. *S. inflata* Dixit (1937).
2. *S. indicum* Singh (1938)
3. *S. ventersicum* Transeau var. *melanosporum* var. nov. Randhawa.

In 1937 Dixit described a new species of *Sirogonium* which he called *S. inflata*, in the course of investigations conducted at the Benares Hindu University. In the following year Singh, from the same Institution, described a new species which he called *S. indicum*. The present author also collected a similar form but with black zygospores from Fyzabad in 1938.

The following comparative chart shows the size of vegetative cells, and the size, shape and sculpturing of zygospores as actually described by Transeau, Dixit, Singh and Randhawa.—

Sl. No.	Character	1	2	3	4
		S. ventersicum Transeau, 1937	*S. inflata* Dixit, 1937	*S. indicum* Singh, 1938	*S. ventersicum* T. var. *melanospora* Randhawa, 1938
1	Size of vegetative cells in microns	65–72 × 110–250	81–99 × 166–298	60–80 × 210–285	80–90 × 140–260
2	Number of chloroplasts	5–8	10	7	6–9
3	Shape of fructifying cells	Inflated	Inflated	Inflated	Inflated
4	Zygospore—				
	Size in microns	80–90 × 133–152	81–85 × 111–139	75–90 × 135–165	90–110 × 140–160
	Shape ..	Ovoid	Ellipsoidal with rounded ends	Ellipsoidal with rounded ends	Ellipsoid
	Sculpturing ..	Irregularly verrucose	Irregularly verrucose	Irregularly scrobiculate	Verrucose

A careful perusal of the above figures will clearly show that the so-called *S. inflata* Dixit and *S. indicum* Singh differ only in very minor details from *S. ventersicum* Transeau. Singh describes the spore-wall of *S. indicum* as *irregularly scrobiculate* which is obviously wrong since it cannot be scrobiculate when it is irregular. It is surprising to note how from the same laboratory two different species, which did not differ materially from an already known species, came to be described in two successive years as two new species.

The present author is of the opinion that *S. inflata* Dixit and *S. indicum* Singh are not valid species and the samples described are covered by the descriptions of the present author's description of *S. ventersicum* Tran. var. *melanosporum*. Black pigment of zygospores usually disappears in preserved material and perhaps due to this reason Dixit and Singh failed to observe it in their material.

A request was made to Dr. Bharadwaja to send some material of the new species of Zygnemales described from his laboratory for verification and comparison. In his letter dated the 3rd April 1942 he writes—" A similar work, as you have intimated to me, is being conducted here on a larger scale in my Department. It would not, therefore, be advisable to allow the same thing to be done at two places."

Consequently the present author could not check the findings of Dixit and Singh had to content himself with their published descriptions and sketches only. There are certain other species like the following which also need to be verified and compared with the already known species. On account of lack of material it is not possible to give any account here.

1. *Zygnema gorakhporense* Singh.—In this case the spore-wall is described as *lamellated with broad scrobiculations* but in the figures the pits are not shown.
2. *Spirogyra crenulata* Singh.
3. *Spirogyra kundænsis* Singh.
4. *Spirogyra Ghoseli* Singh.
5. *Spirogyra anamola* Rao.
6. *Spirogyra bimorphis* Dixit.

Besides the above-mentioned species Rao and Singh have described a number of " formas " of *Sirogyra* and *Zygnema*. While some of these are merely described as " formas ", there are others which are described as '*forma Nov*'. As their names, such as *megaspora, inflata, maxima, crassa* and *tenuis* show, these algæ differ from the type only in larger dimensions of zygospores and vegetative cells, or in having narrower cells and zygospores, or greater or

lesser inflation of fructifying cells, or slight differences in the shape of zygospores varying from ellipsoidal to subspherical. As has been already discussed these differences are of no great value or importance and in most cases are merely of physiological nature. In fact the dimensions of the vegetative cells and zygospores of the same species collected from two different ponds seldom agree. The present author is of opinion that these so-called ' formas ' (which are merely ecological variants) are of little taxonomic value, and do not deserve any special names.

BIBLIOGRAPHY

1. Borge, O. .. "Zygnemales" in *Die Susswasserflora Deutachlan, Odaerstreich und der Schweiz*, 1913, Heft **9.**
2. Carter, N. .. "Freshwater Algæ from India," *Bot. Survey India*, 1926, **9,** 263–302.
3. Chesterman, D. R., and Forster, C. L. "Creeping movements of *Spirogyra*," *Nature*, 1936, **2,** 1923.
4. Chodat, R. .. "Etudes Sur les Conjuguees. I Sur La copulation d'un Spirogyra," *Bull. Soc. Bot. Geneva*, 1910, Ser. 2, **2.**
5. Cholonoky, B. V. .. "Einige Bemerkungen zur Zygotenbildung der Conjugaten," *Arch. f. Protistenk*, 1929, **65.**
6. ———— .. "Vergleichende Studien Uber Kern- und Zellteilung der fadenbildenden Conjugaten," *ibid.*, 1932, **78.**
7. Czurda, V. .. "Zygnemales" in *Die Susswasserflora Mittleleuropas*, 1932, Heft 9, **27.**
8. ———— .. "Zur Morphologie and Systematik der Zygnemalen," *Beith Bot. Centralbl.*, 1931, **38,** 238–85.
9. ———— .. "Eni neuer engenartiger Copulationsaplauf bei einer *Mougeotia* M. Oedogonioides Czurda," *ibid.*, 1931, **38,** 286–90.
10. ———— .. "Conjugatæ," *Handbuch der Pflanzenanatomie*, Band **6,** Berlag von Gehruder Borntræger, Berlin, 1937.
11. Delf, E. M. .. "Note on some attached species of *Spirogyra*," *Ann. Bot.*, 1913, Bd. **27.**
12. Dixit, S. C. .. "The Chlorophyceæ of the Bombay Presidency, India, I," *Proc. Ind. Acad. Sci.*, 1937, B **5,** 16–25.
13. Fritsch, F. E. .. *The British Freshwater Algæ*, Camb. University Press, 1932.
14. ———— .. *The Structure and Reproduction of the Algæ*, Camb. University Press, 1935, **1.**
15. ———— .. "The morphology ad ecology of an extreme terrestrial form of *Zygnema* (*Zygogonium*) *ericetorum* (Kutz.)," *Ann. Bot.* 1922, **30.**
16. ——— and Rich, F. .. "The reproduction and delimitation of the genus *Zygnema*," *New Phytol.*, 1927, **26.**
17. ———— .. "Freshwater and sub-aerial Algæ from Natal," *Trans. Roy. Soc. of S. Africa*, 1929, **11.**

Gau, B. .. *Reitrage Zur Morpholigie and Biologie von* Zygogonium ericetorum *Diss.*, Konigsberg, 1934.

19. Handa, M. R. .. "Some peculiar features of sub-aerial *Zygnemales* of Rangoon," *Jour. Ind. Bot. Soc.*, 1927, **6.**

20. Hodgetts, W. J. .. "A new species of *Spirogyra*," *Ann. Bot.*, 1920, **34.**

21. Iyengar, M. P. O. .. "Note on some attached forms of Zygnemaceæ," *Jour. Ind. Bot. Soc.*, 1923, **3.**

22. ———— .. "Studies on Indian Zygnemales," *Rev. Algol.*, 1932, **6.**

23. ———— .. *Presidential Address*, 15*th Ind. Sci. Cong., Sec. Bot.*, 1932.

24. Jao, C. C. .. "New Zygnemaceæ collected in China," *American Journ. Bot.*, 1923, **3.**

25. ———— .. "Zygnemataceæ from Szechuan," *Sinenssia*, 1935, **6**, No. 5.

26. ———— .. "New Zygnemataceæ from Woods Hole," *Trans. American Micros. Soc.*, 1935, **54**, 1.

27. Kolkwitz, R., und W. Krieger "Zur Okologie der Pfanzenwalt, inbesondere der Algen, des Vulkans Pangerango in West Java," *Ber. Deutsch. Bot. Gas.*, 1936, **44**, 65–91.

28. Li Liang Chiang .. "New species and varieties of Freshwater algæ from China," *Ohio Jour. Sci.*, 1933, **33**, 151–55.

29. ———— .. "A contribution to the Freshwater Algæ of Kiangsi," *Bull. Trans. Inst. Biol.*, Bot. Ser., 1938, **8**, 65–112.

30. Lloyd, Fr. E. .. "Maturation and conjugation in *Spirogyra longata*," *Trans Royal Inst. Toronto*, 1926, **15.**

31. Merriman, M. L. .. "Nuclear division in *Zygnema*," *Bot. Gaz.*, 1906, **41.**

32. ———— .. "Studies in the conjugation of *Spirogyra ternata*," *Bull. Torr. Bot. Club*, 1920, **47.**

33. Misra, J. N. .. "Zygnemataceæ of Kashmir, I," *Proc. Ind. Acad. Sci.* (B), **5**, No. 3.

34. Oltmanna, F. .. "Morphologie und Biologie der Algen," Jena, 1923.

35. Randhawa, M. S. .. "*Ghosella indica*—A new member of Conjugatæ," *Jour. Ind. Bot.*, 1934, **13**, No. 1.

36. ———— .. "Marked periodicity in Reproduction of the Punjab Freshwater Algæ," *Proc. Ind. Acad. Sci.*, B, 1936, **3**, No. 5.

37. ———— .. "Occurrence and distribution of the Freshwater Algæ of North India," *ibid.*, 1936, **4**, No. 1.

38. ———— .. "Three new species of *Zygnema* from India," *ibid.*, 1936, **4**, No. 3.

39. ———— .. "A note on some Attached Forms of *Spirogyra*," *ibid.*, 1936, **4**, No. 3, 246–49.

40. ———— .. "Genus *Zygenmopsis* in Northern India," *ibid.*, 1937, **6**, 297–314.

41. ———— .. "Observations on Some Zygnemales from Northern India, Parts I and II," *ibid.*, 1938, **8**, 109–50.

42. ———— .. "*Zygnema terrestris* Randh. from the Kumaon, Himalayas," *Curr. Sci.*, 1940, **9**, 373.

43. ———— .. "Some peculiarities in conjugation in a new Himalayan species of *Zygnema*," *Proc. Ind. Acad. Sci.*, 1940, **12**, 129–32.

44. ———— .. "*Zygogonium kumaœnsis*, a new species of *Zygogonium* from Kumaon," *Jour. Ind. Bot. Soc.*, 1940, **19**, 247–49.

45. Randhawa, M. S. .. "*Sirocladium*, a new terrestrial member of the Zygnemales," *Bot. Gaz.*, 1941, **103**, 129–97.

46. Rao, C. B. .. "The Zygnemoideæ of the United Provinces, India, I," *Jour. Ind. Bot. Soc.*, 1937, **16**, 267–88.

47. Saunders, H. .. "Conjugation in *Spirogyra*," *Ann. Bot.*, 1931, **45.**

48. Singh, R. N. .. "The Zygnemoideæ of the United Provinces, India, II," *Jour. Ind. Bot. Soc.*, 1938, **17**, 368–84.

49. Smith, G. M. .. *Fresh-water Algæ of the United States*, Mc-Graw Hill Book Co., 1933.

50. Skuja, H. .. "Zwei New Zygnemaceen mit blanem mesospore," *Acta Horti. Bot. Univ. Lat.*, 1926.

51. ———— .. "Susswasseralgen von den Westestnischen Inselu Saaremaa and Hiiumao," *ibid.*, 1928, U. Bd. **4.**

52. ———— .. "Vorarbeiten zu einer Algenflora von Lettland, IV," *ibid.*, 1928, **3.**

53. ———— .. "Susswasserlagen aus Griechenland und Kleinasien," *Hedwigia*, 1937, 77, 15–70.

54. Tiffani, L. H. .. "Some New Forms of *Spirogyra* and *Oedogonium*," *Ohio Jour. Sci.*, 1924, 34.

55. ———— .. *Ibid.*, 1924, 24.

56. Toni, de, J. B. .. *Sylloge Algarum Padua*, 1889, Bd. **6.**

57. Transeau, E. N., Tiffani, L. H., Taft, C. E., and Li, L. C. "New species of Zygnemataceæ," *Trans. American Micros. Soc.*, 1934, **53**, 208–80.

58. Transeau, E. N. .. "New species of Green Algæ," *American Jour. Bot.*, 1914, **1**, 189.

59. ———— .. "Notes on the Zytnemales," *Ohio Jour. Sci.*, 1916, 16, 17.

60. ———— .. "Genus Debarya," *ibid.*, 1925, 25, 193.

61. ———— .. "The Genus Temnogametum," *ibid.*, 1932, **32**, 235–385.

62. ———— .. "The Genus Mougeotia," *ibid.*, 1933, 26, 311.

63. ———— .. "The Genera of the Zygnemataceæ," *Trans. American Micros. Soc.*, 1934, **53**, 201.

64. ———— .. "Preliminary Key to the Species of Zygnematales," *Mimeographed Manuscript*, 1934.

65. ———— .. "Notes on Zyenemataceæ," *American Jour. Bot.*, 1938, **25**, 524–28.

66. Turner, W. B. .. "Algæ aquae dulcis Indiae Orientalis," *K. Sv. Vet. Akad. Handl.*, 1892, **25.**

67. Uspenski, E. E. .. "Transformation of certain hereditary forms into other ones and the arising of new forms in the Genus *Spirogyra*," *Microbiol., Moscou*, 1934, **3.**

68. West, G. S. .. "The Algæ of the Yan Yean Reservoir, Vistoria, a Biological and Ecological Study," *Jour. Linn. Soc.*, 1909, **39**, 1.

69. ——— and West, Wm. .. "Observations on the Conjugateæ," *Ann. Bot.*, 1898, **12.**

70. West, W., and West, G. S. "A Contribution to the Fresh-water Algæ of Ceylon," *Trans. Linn. Soc. London*, 1902, **6**, 123.

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CONTRIBUTIONS TO OUR KNOWLEDGE OF THE PYLORIC CÆCA IN THREE FAMILIES OF FRESH-WATER INDIAN FISHES (OPHICEPHALIDÆ, NOTOPTERIDÆ AND MASTACEMBELIDÆ), TOGETHER WITH SOME REMARKS ON THEIR PROBABLE FUNCTIONS

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With 1 Plate and 4 Text-Figures

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1. *Introductory*

REFERENCES to up-to-date literature revealed that so far no regular work has been attempted on the pyloric cæca in the fresh-water fishes of India, and it is for this reason that my former teacher and colleague, Professor

The term pyloric cæca is retained throughout, though actually these structures arise from the duodenum.

B. K. Das of the Osmania University, suggested to me that it would be worthwhile to work out fully and to make a thorough comparative and systematic study and survey of these organs (and later on their physiology) in fishes of our own waters. From certain stray accounts it has been gathered that these structures have just been casually mentioned to be present in some 31 families of Indian fishes, including nearly 76 genera and about three times as many species, most of which are marine, some estuarine and a few fresh-water forms.

After consulting the relevant literature one would find that the structure (and to some extent the physiology) of the pyloric cæca has been briefly described by a handful of workers only, amongst which the most notable ones are Rosenthal (1824), Hyrtl (1864), Blanchard (1882), Stirling (1884), Fr. Day (1887), Bondouy (1897–99), Johnson (1907), Kostanecki (1913) and Dharmarajan (1936), whose works have been reviewed in this paper. Acting on Professor Das's suggestion I have first of all worked out the structures of these cæca in four species of the family Ophicephalidæ, and then one of Notopteridæ and three of Mastacembelidæ, thus making a total of eight species of fresh-water fishes commonly met with in Hyderabad, and the results of nearly a year's work of mine are embodied in the following pages.

2. *Historical Summary*

There are but a few small papers dealing with the account of the pyloric cæca in teleostean fishes:

In 1824 Rosenthal has just touched upon the condition of the pyloric cæca in the Sword-fish without giving any figure. Hyrtl (1864) has shown a very curious disposition and the mode of opening of the bile duct actually into the " appendices pyloricæ " in *Fistularia*, *Aulostoma* and *Acanthurus*—there being a single cæcum in *Fistularia*, two in *Aulostoma* and six in *Acanthurus*. The account given by Johnson (1907) is, however, an interesting one in which he has referred to " The individuality and variation of the pyloric cæca of the Centrarchidæ". The main object of his contribution is just to show that the pyloric cæca of certain members of this particular family, *viz.*, the Sun-fish, *Lepomis*, Black Bass, *Micropterus*, etc., are not similar, as generally assumed, but that they differ considerably in number and form: in other words, they show a lot of individual variations in a single family, that is to say, the pyloric cæca have an individuality of their own in every species of fish. Here the cæca vary in number, usually from 6 to 19, but in *Micropterus* they may be considerably branched, and these branches may be as many as 28.

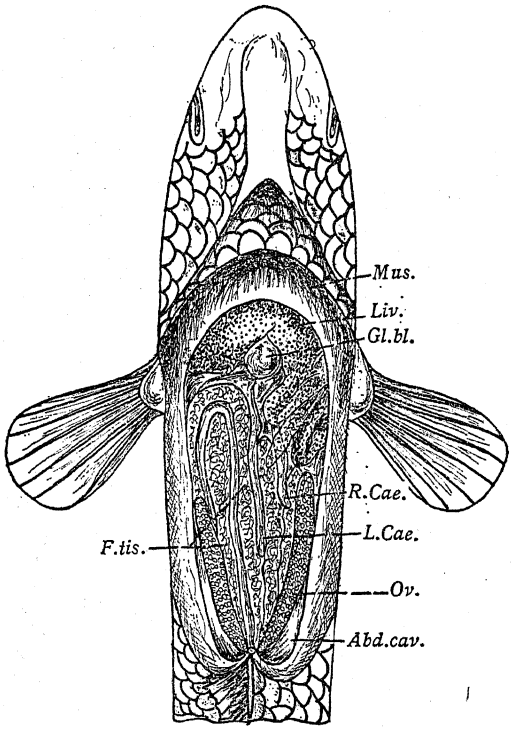
3. *Material and Technique*

In every case fresh material for histological study was obtained after pithing the live fish. In order to have a perfect fixation and preservation, both internally and externally, the two pyloric cæca were first of all injected with Bouin's fluid until they were quite turgid from the œsophageal side with the help of a small syringe after cutting off the œsophagus and inserting the nozzle at its distal end, and then finishing off the operation by giving two ligatures after fluid had run inside the whole length of the alimentary canal quite satisfactorily—one knot was tied at the remote end of the œsophagus and the other at the proximal end of the ileum slightly behind the cæca. The cæca and the associated parts of the gut were then removed and preserved in the same fluid for 8–24 hours. After having carefully dissected out the cæca from the surrounding tissues, each, as a rule, was cut into three portions, *viz.*, the *proximal*, the *middle* and the *distal* segments (except in the case of the Mastacembelidæ, in which the two cæca, being very small and very closely situated together, have been treated as a whole). Each piece of the cæca as also other parts of the alimentary canal were imbedded separately in paraffin blocks, and serial sections (both transverse and longitudinal), from 6 to 8 μ thick, were cut. They were variously stained, such as, for instance, in picro-indigo-carmine, Mallory's triple, Heidenhain's iron-hæmatoxylin, and Delafield's hæmatoxylin counterstained with eosin. Several freehand and camera lucida sketches were made, and many photomicrographs have also been taken and compared.

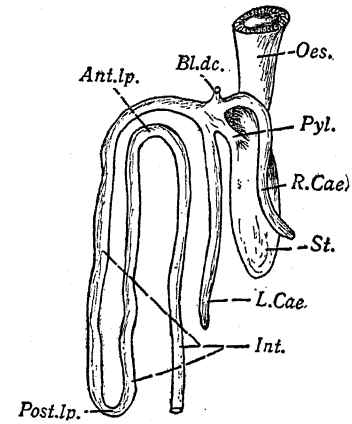
It is my most pleasant duty to record my sincere thanks here to Professor Dr. A. Subba Rau, B.A., D.Sc. (London), F.R.M.S., Principal and Head of the Zoology Department of the Central College, Bangalore, for his kindly going through the MS. and making some very useful suggestions as well as for accepting the paper for publication in this journal. I am very grateful to Professor R. Gopala Aiyar, Director of the Madras University Zoological Research Laboratory, for his kind help and friendly criticisms. I am also grateful to Professor B. K. Das for his help and guidance. I am very thankful to Professor A. B. Misra of the Benares Hindu University for certain valuable advice.

4. *The Pyloric Cæca of* Ophicephalus striatus *Bl. as a Type*

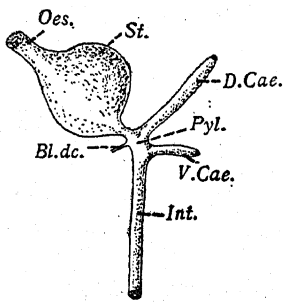
(*a*) *Topography* and *Morphology*.—As an example of the typical condition of the pyloric cæca, mention may first of all be made of *Ophicephalus striatus* Bl., the "Murrel" (the second largest member of the Fam. Ophicephalidæ), a fish which is very commonly found in Hyderabad. In this fish, there are two fairly large cæca (right and left, Text-Fig. 1, *a* and *b*,



TEXT-FIG. 1 (*a*)

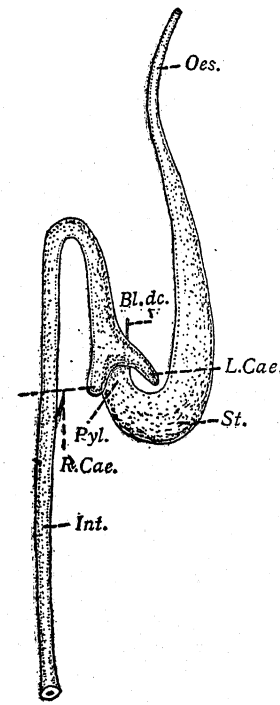


TEXT-FIG. 1.(*b*)



TEXT-FIG. 2

TEXT-FIG. 4



TEXT-FIG. 3

Text-Fig. 1 (*a*). Dissection of *Ophicephatus striatus* from the ventral aspect, showing the pyloric cæca *in situ* (× 1½). 1 (*b*). Alimentary canal of the same fish unravelled, showing the disposition of the cæca (× 1½). Text-Fig. 2. Anterior portion of the alimentary canal of *Notopterus notopterus* unravelled, showing the disposition of the cæca (× 2). Text-Fig. 3. Alimentary canal of *Mastacembelus armatus* unravelled, showing the disposition of the cæca (× 2). Text-Fig. 4. Ditto of *Fistularia villosa*, showing the disposition of the *single* cæcum (× 3).

R. cæ. and *L. cæ.*) which are tubular, digitiform structures arising just from the commencement of the small intestine; that is to say, immediately behind the pylorus (*Pyl.*) which is quite short. The bile-duct (*Bl. dc.*) often opens immediately behind the origin of the right cæcum, or sometimes in the very narrow interspace between the right and the left cæca. The right cæcum is usually somewhat smaller in size than the left one. The intestinal orifice of each cæcum is quite distinct though very small, but, unlike that of the rectal gland of Selachian fishes where there is a valve, it is unguarded by any valvular structure.

The cæca are invariably filled with some semi-digested food-material mixed up with a small quantity of mucous, and in certain rare cases some bile also. There is a large amount of fat (*F.tis.*) that conceals the greater portion of these cæca.

The intestine of this carnivorous fish, as one would naturally expect, is of a simple type and bears two loops—one at the anterior end (*Ant. lp.*) and the other at the posterior end (*Post. lp.*). Further, it is interesting to compare the relative lengths of the various parts of the alimentary canal with those of the cæca, and the ratios may be stated as follows:—

Cæca : Intestine, and Cæca : whole length of the alimentary canal $= \frac{1:3\cdot9\ \mathrm{R}}{1:3\cdot2\ \mathrm{L}}$ and $\frac{1:5\cdot2\ \mathrm{R}}{1:4\cdot2\ \mathrm{L}}$ respectively, where R denotes the right cæcum and L the left cæcum, and the length of the cæcum (either of the right side, or of the left side) has, in each case, been taken to represent as a unit.

(*b*) *Blood- and Nerve-Supplies.*—The blood-supply is quite interesting in a way that the cœliaco-mesenteric artery arises from the right side of the dorsal aorta and divides into three main branches :—

(1) Gastric artery going to the stomach.

(2) Cæcal artery bifurcating into two smaller twigs, and supplying the right and the left cæca.

(3) Anterior mesenteric artery supplying the proximal part of the intestine.

The blood is returned from these cæca by means of two factors, *viz.*,

(1) The cæcal vein draining blood from the right and left cæca and emptying itself into the Hepatic Portal vein. Into each of these cæcal factors veins from the stomach also open.

(2) A small vein arising independently from the right cæcum and dipping into one of the tributaries of the Hepatic Portal vein.

As regards the nerve-supply it is worthy of note that the visceral branch of the *right* Vagus divides into four small branches, *viz.*, the cardiac, gastric, intestinal and cæcal, supplying the heart, stomach, intestine and the two cæca respectively, whereas the *left* visceral has no cæcal branch, but in other respects it is exactly like its right counterpart.

(*c*) *Histology.*—After a careful examination and study of a large series of transverse and longitudinal sections of the pyloric cæca the following histological details add a great interest to our knowledge, and may be briefly mentioned thus—here the figures of *Ophicephalus marulius* Ham. have been given merely for the sake of convenience :—

(1) Roughly speaking, the internal structure of the proximal region of the pyloric cæca (Pl. IV, Fig. 2) is pretty similar to and built upon the same general plan as that of the small intestine (Pl. IV, Fig. 1).

(2) The cæcal villi (Pl. IV, *Cæ. vil.*, *i.e.*, the folds of the mucosa of the cæcum) are, as a rule, very prominent and extend for a considerable distance inside the cæcal lumen.

(3) The various layers which compose the wall of the cæcum, from without inwards, are (Pl. IV, Fig. 2) :—

(i) Serous coat = visceral peritoneum (*Ser.*).

(ii) Layer of longitudinal muscle-fibres (*Long. musc.*) = thin envelope.

(iii) Layer of circular muscle-fibres (*Circ. musc.*) often twice or three times as thick as No. (ii).

(iv) Sub-mucosa (*Sub. muco.*) consisting of connective-tissue, some blood-capillaries (*Bl. cap.*) and nerve fibres.

(v) Mucosa usually consisting of a two- or three-cell deep layer, made up mostly of columnar or stratified epithelial cells amongst which a large number of goblet cells are also to be found. It is thrown into a very large number of deep folds which form finger-like structures (or the " cæcal villi ", *Cæ. vil.*) penetrating into the cæcal lumen—the central core of each " cæcal villus " is highly vascular, having many fine blood-capillaries which traverse and extend up to its distal end, and probably thus increase the absorptive surface of the cæcum.

Curiously enough, in the *distal* region of each cæcum (Pl. IV, Figs. 3 & 5) the " cæcal villi " grow in size and multiply tremendously, interdigitate and fuse with one another thus presenting the appearance of a sort of spongy structure (or what has been designated here as " spongy-tissue ", *Int. dig.*

muc. fl.) filling up and practically obliterating the whole of the cæcal lumen (*Cæ. lum.*) in other words, this sort of excessive branching and highly folded arrangement of the "cæcal villi" evidently provides greater area for the absorption of the digested soluble food in this region of the cæcum.

5. Condition of the Cæca in Some other Fresh-Water Indian Fishes

(A) *Other Species of the Family Ophicephalidæ* :

In the other three species of the family Ophicephalidæ which have been investigated, *viz.*, *Ophicephalus marulius* Ham. (the largest species), *O. punctatus* Bl., *O. gachua* Ham. (the smallest species), the general structure of the cæca (together with their blood- and nerve-supplies) is practically just the same as that described for *O. striatus* Bl., but there are a few minor variations which may be very vriefly pointed out as follows :—

(1) The pyloric cæca are club-shaped in *O. punctatus*, whereas they are of a tapering nature in *O. gachua*.

(2) The various ratios of the cæca for the other three species, as those described above for *O. striatus*, are :—

(i) *O. marulius* $= \frac{1:6{\cdot}2\ \text{R}}{1:5{\cdot}2\ \text{L}}$ and $\frac{1:8{\cdot}7\ \text{R}}{1:7{\cdot}3\ \text{L}}$ respectively,

(ii) *O. punctatus* $= \frac{1:6{\cdot}6\ \text{R}}{1:4{\cdot}8\ \text{L}}$ and $\frac{1:8{\cdot}6\ \text{R}}{1:6{\cdot}2\ \text{L}}$ respectively, and

(iii) *O. gachua* $= \frac{1:7{\cdot}1\ \text{R}}{1:5{\cdot}7\ \text{L}}$ and $\frac{1:8{\cdot}5\ \text{R}}{1:6{\cdot}8\ \text{L}}$ respectively.

(R and L, wherever being used, always signify right and left cæca as in the case of *O. striatus*.)

(3) The "cæcal villi" of *O. punctatus* are relatively very large—they are nearly twice the size of the "intestinal villi".

(4) The simplest type of "cæcal villi" are to be found in the smallest species of this family, *viz.*, *O. gachua*, in which they do not multiply and fuse together in the distal region of the cæcum (Pl. IV, Fig. 4) as they do in all other species of the Ophicephalidæ as well as in most other fishes that I have studied so far.

(B) *Notopterus notopterus* (Pallas):

(*a*) *Topography and Morphology*.—In this fish the whole of the alimentary canal is relatively very short (Text-Fig. 2). The intestine (*Int.*) lies below the large gas-bladder and is thrown into a semi-circular loop with the

convex surface directed upwards. There are two curved conical cæca,* one of which is dorsal in position (*D. cæ.*), and the other ventral (*V. cæ.*), the former being bigger than the latter, and both lying hidden between the globular stomach (*St.*) and the intestine (*Int.*), and running closely parallel to the latter. The pylorus (*Pyl.*) is very small.

The ratio of the lengths of the *dorsal* and the *ventral* cæca : intestine = 1 : 2·5 and 1 : 4 respectively (the length of either of the cæca being taken as a unit). Ditto dorsal and ventral cæca : whole length of the alimentary tract = 1 : 3 and 1 : 5 respectively.

(*b*) *Blood- and Nerve-Supplies.*—(1) The cœliaco-mesenteric artery gives off three branches :—

(i) Intestinal—supplying the whole of the intestine.

(ii) Gastric—supplying the stomach and also sending a small branch to the posterior part of the dorsal cæcum.

(iii) Cæcal artery divides into two branches : one going to the dorsal cæcum and the other to the ventral.

(2) The blood is drained from the cæca by two ways :

(i) Two cæcal veins bring back blood independently from the dorsal and the ventral cæca and ultimately join the Hepatic Portal vein.

(ii) Small veins draining blood from the posterior ends of both the cæca and falling into the intestinal factor of the Hepatic Portal.

As regards the nerve-supply it may be mentioned that the visceral branch of the *left* Vagus, unlike the previous case, innervates the stomach and the two cæca, whereas its *right* counterpart sends off twigs to the intestine and the dorsal cæcum only.

(*c*) *Histology.*—The salient features in the histology of the cæcum of *Notopterus* as distinguished from those of the ophicephalids are as follows :

(1) Elongated digitiform "cæcal villi" and several large goblet cells are present in the cæcal epithelium towards the *Proximal* and the *Mid*-regions of the cæcum.

(2) In the *distal* region of the cæcum, however, the "villi" do not proliferate so copiously as they do in the Ophicephalidæ, but most of them penetrate inwards towards the centre of the cæcal lumen, and some of them also unite with one another (*Int. dig. muc. fl.*—Pl. IV, Fig. 5), forming many narrow inter-communicating passages or channels (*Ch.*) inside the cæcal

* In this figure the cæca are displayed after being straightened out.

lumen—all these modifications are evidently meant to increase the absorptive surface of the cæcum.

(C) *Various Species of the Family Mastacembelidæ:*

Three members of this family, *viz.*, *Rhynchobdella aculeata* (Bloch), *Mastacembelus armatus* (Lácep.) and *M. pancalus* (Ham.) have been studied, but here I shall confine myself in describing the condition in *Mastacembelus armatus* (Text-Fig. 3) as a typical case, which agrees with the other two species practically in all essential respects.

(*a*) *Topography and Morphology.*—In *M. armatus* (as also in the other two species of this family) there are two cæca (right and left, Text-Fig. 3, *R. cæ.* and *L. cæ.* respectively) which are relatively very small as compared with those of *Notopterus notopterus* (Pall.) and the several species of the Fam. Ophicephalidæ. They are short, stumpy, finger-like structures originating from the junction of the duodenum with the pylorus (*Pyl.*) and to some extent adhering to the sides of the latter. They are practically equal in size, but sometimes the left cæcum may be just slightly bigger than the right one.

The ratio of the length of any one of the two cæca (right or left—both being regarded to be of equal size): intestine = 1: 14·6, and the cæcum: whole length of the alimentary tract = 1: 25·0 (length of either cæcum representing a unit as in previous cases).

(*b*) *Blood- and Nerve-Supplies.*—(1) The cœliaco-mesenteric artery gives off 4 branches:

(i) Gastric—supplying the stomach.

(ii) Intestinal—supplying the intestine.

(iii & iv) Two independent cæcal arteries, and supplying the right and the left cæca.

(2) The blood is returned from both the right and the left cæca by a single cæcal vein which joins the intestinal factor of the Hepatic Portal vein.

Regarding the nerve-supply it may be mentioned that the *right* visceral branch of the Vagus, as in the Ophicephalidæ, sends off small branches to both the cæca, the stomach and the intestine, whereas its *left* counterpart mainly innervates the stomach and the mesentery.

(*c*) *Histology.*—In the *distal* region of the pyloric cæca of the Fam. Mastacembelidæ the "cæcal villi" (Pl. IV, Fig. 6) are comparatively more developed than in the Notopteridæ: here they form a lot of infoldings and interdigitation and are all compacted together, having the " villi " massed up side by side in a slightly oblique manner, and roughly presenting the appearance

of a small gland. Not infrequently, however, the mucous folds also unite with one another, chiefly towards the posteriormost part of the cæcum, as observed in other fishes, thus increasing its absorptive surface. Another noteworthy fact is that only a few goblet cells are present along the lumen epithelium of this group.

As far as histological structures are concerned in the other two species, *viz.*, *M. pancalus* and *R. aculeata*, it is worthwhile to remember that there is no deviation from the typical condition as just described above in the case of *M. armatus*.

6. *Discussion*

Here, I will just deal very briefly with the nature and significance of the pyloric cæca in a summarised form as they exist in various groups of fishes in general :

(1) The pyloric cæca are absent in Cyclostomata, Dipnoi, and practically in all Elasmobranchs, but there is a considerable variation as to the number, form and structure of these cæca in various members of the Teleostomi, some of which are already described in most of the text-books on fishes. A very good summarised comparative account of these cæca in fishes, found outside India, is given in *Handbuch der Vergleichenden Anatomic der Wirbeltiere*, Vol. III (1937), by Pernkopf, Lehner and Jacobshagen.

(2) For instance, amongst the Ganoids the pyloric cæca are absent in the Bow-fin (*Amia*). In the " Bichir " (*Polypterus*) there is a single cæcum whereas in other members of this group, *viz.*, Sturgeon (*Acipenser*), Spoonbill (*Polyodon*), and Gar-pike (*Lepidosteus*) the cæca are very well developed.

(3) Again, in certain groups of Teleosts, the cæca are entirely absent as, for example, in the Cat-fishes (Siluridæ), Pikes (Esocidæ), Toothed-carps (Cyprinodontidæ), Wrasses (Labridæ), Plectognathi including the Globe-fishes, the Porcupine-fishes and lastly in the Pipe-fishes (Syngnathidæ).

(4) Whilst in some others, including both the European as well as the Indian types, the cæca may be very numerous (*i.e.*, at least more than 50) as, for example, in Salmon (*Salmo*), whiting (*Gadus merlangus*), Mackerel (*Scomber scombrus*), and certain Clupeidæ. They may be many in number as in the "White Pomfret" (*Stromateus sinensis*), *Sphyræna*, the "Hair-tail" (*Trichurus*), the "Pompano" (*Caranx*), etc. ; moderate number (*e.g.*, 16) in the so-called "Bombay Duck" (*Harpodon*); 5–7 in *Acanthurus* ; 3–5 in certain Pleuronectids ; only a few (3, for example) in *Premnas* and *Tetradrachmum* (Fam. Glyphidodontidæ) ; two in *Notopterus*, Ophicephalids, Sand-eels (*Mastacembelidæ*), the "Gourami"; (*Osphronemus*), etc.;

and only one in *Fistularia villosa* (Fam. Fisturlariidæ, Text-Fig. 4), and besides these, there are, of course, several other genera and species that are not mentioned here—it would be too cumbersome a list to deal with all of them within the limited space at present.

(5) It is also very interesting to note that in certain extreme cases, *e.g.*, Sturgeon, Whiting and Tunny (*Thunnus thynnus*) where the cæca are not only numerous, but most of them (or in some cases, all of them) are also united together by means of connective tissue to form a compact, gland-like mass communicating with the intestine, either by a *single* wide duct (as in Sturgeon) or by *several* small orifices as in other examples. Such a condition of the pyloric cæca would naturally lead to the assumption that probably they have some sort of secretory function, supplementing the actions of the digestive glands, such as the liver and the pancreas. At any rate one might say that such cæca must be of some important use in connection with the digestive functions of the fish in which they occur and have assumed such a compact, gland-like character. In other words, in such cases they may be said to represent acessory digestive glands.

7. *Summary and General Conclusions*

(1) The pyloric cæca are true outgrowths of the *proximal* portion of the small instestine (*i.e.*, the duodenum), as has been corroborated by their histological structure, and hence the name pyloric cæca is really a misnomer: the correct name for them should be " intestinal cæca ".

(2) They should not be mixed up with the " cæcal " or " rectal gland " of Selachian fishes, which has been so thoroughly worked out by Miss D. R. Crofts in recent years (*cf.* *P.Z.S.*, 1925). *That is to say they are not homologous with the " cæcal " (or " rectal gland ") of Selachian fishes, nor with any other cæcal outgrowth of other vertebrates, because all such latter structures take their origin between the large and the small intestine, whereas the pyloric cæca are given off immediately behind the pylorus as true outgrowths of the first part of the ileum.*

(3) From the presence and nature of the semi-digested liqud food-contents inside the lumen of the pyloric cæcum and the opening of the latter into the ileum as well as in due consideration of a fairly large amount of vascular supply (particularly the drainage of the blood into the portal system) and also in due recognition of the significance of the very structure of those copiously distributed digitiform " cæcal villi ", comparable to the true intestinal villi, the following physiological functions may possibly be attributed to them:—

(*a*) Might serve as accessory food-reservoirs in these fishes—the intestine in most of these fishes being of shorter lengths (so far investigated), but this fact could not be generalised yet until a very large number of fishes has been thoroughly examined.

(*b*) Probably a part of digestion might take place.

(*c*) Some absorption of the digested food may probably also take place (*cf.* from the nature of the highly vascular "cæcal villi").

(*d*) According to some authors (Mordacai, 1882; Blanchard, 1882; Stirling, 1884; and Bondouy, 1897 and 1899), who have worked on the physiology of pyloric cæca in certain other fishes, the following probable functions have been assigned to them:

That they are said to produce diastatic and trypsin-like enzymes which effect some digestion of the carbohydrates and the proteids, and thus help and supplement the digestive processes of other juices poured into the alimentary canal. (I have, however, no sufficient physiological data at my disposal just at present to fully test and justify the validity of this statement and, at any rate, I am presently engaged in carrying on a series of physiological experiments and biochemical tests on the contents of the pyloric cæca, and my results will shortly be communicated in later papers.

(*e*) It is not yet quite certain if diet has really any marked effect or influence on the relative size and structure of these cæca, and this point can only be definitely settled when a very large number of fishes, belonging to various families and living in different environment (and having different diet) has been thoroughly investigated, especially from this point of view—at this stage, in absence of any further data, it would rather be too hazardous to speculate anything.

(*f*) It is also very doubtful whether the number and nature of the pyloric cæca are of any taxonomic value in the study of fishes—this point could also be elucidated after a thorough systematic examination of a very large number of different species and families of fishes with which I am engaged at present.

REFERENCES TO LITERATURE

Anile, A. .. "Contrabatio alla conceaza della piloriche nei Teleostei," *Publicazione Staz. Zool. Napoli*, 1918, **2**.

Blanchard, R. .. "Sur les fontions des appendices pyloriques," *C. R. Acad. Sci.*, Paris, 1882.

Bondouy, Th. .. "Sur les phénomènes digestifs dans les tubes pyloriques chez quelques téléostéens," *Bull. Soc. Scient. Med. Quest.*, 1897.

——— .. "Du rôle des tubes pyloriques dans la digestion chez la téléostéens," *Arch. Zool. Expre.*, 1899.

Bondouy, Th. .. "Recherches sur la valeur physiologique des tubes pyloriques de quelques téléostéens," *C. R. Acad. Sci.*, Paris, 1899.

Bridge, T. W. .. *Fishes*, C. N. H. Series, 1922.

Crofts, D. R. .. "The Comparative Morphology of the Cæcal Gland (Rectal Gland) of Selachian Fishes, with some references to the Morphology and Physiology of the similar Intestinal Appendages throughout Ichthyopsida and Sauropsida," *P.Z.S.*, 1925, Part I, 101–88.

Dawes, Ben .. "The Histology of the Alimentary Tract of the Plaice (*Pleuronectes platessa*)," *Q.J.M.S.*, 1930, **73**, No. 290.

Day, F. .. "Notes on Cæcal appendages of fresh-water Trout," *Journ. Nat. Fish. Cult. Assoc.*, 1887.

———— .. "Notes on the Cæcal appendages of Cardigenshire Trout," *Ibid.*, 1887.

———— .. *Fishes, Fauna Brit. Ind.*, 1889, **1** and **2**.

Del vecchio, C. .. "Richerche sulla struttura dello stomaco dei pesci con speciale righuardo alla parte pilorica," *Atti. Soc. Ital. Sci. Nat.*, 1917, **56**.

Dharmarajan, M. .. "The Anatomy and Histology of the Alimentary System of *Otolithus ruber* (Bl. Schn.)," *Proc. Ind. Sc. Cong.*, 1936.

Goodrich, E. S. .. "Cyclostomes and Fishes", *A Treatise on Zoology* (Lancester Series), 1909.

Hyrtl, C. J. .. "Über die Einmundung des Ductus choledochus in eine Appendix pyloricæ," *Sitzber. Akad. Wiss.*, Wien, 1864.

Jacobshagen, E., Pernkopf, E., and Lehner, J. .. *Handbuch der Vergleichenden Anatomie der Wirbeltiere*, 1937, **3**.

Johnson, R. H. .. "The individuality and variation of the pyloric cæca of the Centrarchidæ," *Trans. Wiscon. Acad. Sci.*, 1907.

Kostanecki, K. .. "Zur Vergleichenden Morphologie des Blinddärmes unter Berücksichtigung seines Verhältnisses zum Bauchfell," *Anat. Hefte*, 1913.

Kyle, H. M. .. *The Biology of Fishes*, 1926.

Norman, J. R. .. *A History of Fishes*, 1931.

Oppel, A. .. *Lehrbuch der vergleichenden mikoskopischen Anatomie der Wirbeltiere*, 1896–1900, **1–3**.

Owen, R. .. *Comparative Anatomy and Physiology of Vertebrates*, London, 1886, **1–3**.

Panicker, M. K. N. .. "Histology of the Alimentary tract of *Therapon quadrilineatus*," in MS.

Rahimulla, M. .. "A contribution to the Structure and Probable Functions of the Pyloric Cæca in the Fam. Ophicephalidæ," *Anat. Anz.*, **Bd.** 1935, **80**, Nr. 1/4.

———— .. "On the Structure of the so-called Pyloric Cæca in a Marine Genus *Platycephalus*," *Proc. Nat. Acad. Sci., Ind.*, 1941, **2**.

———— .. "On the Structure and Functions of the so-called Pyloric Ceaca in the two genera of fishes, *Lactarius* and *Osphronemus*," *Journ. Osm. Univ.*, 1941.

Riggio, G. .. "Sopra un caso di notevole ramificazione dei ciechi pilorici di *Centrolophus pompilus* Cuv. et Val.," *Nat. Sicil*, 1894.

Rosenthal, F. C. .. "Über die Blindärme im Schwertfische," *Ztschr. Physiol.*, 1824.

Stirling, W. .. "On the chemistry and histology of the digestive organs of fishes," *Rept. Scot. Fisheries*, 1884.

EXPLANATION OF PLATE

Fig. 1. Camera lucida sketch of a part of the transverse section of the intestine of *Ophicephalus marulius*, showing the very prominent "intestinal villi".

Fig. 2. Ditto of the *proximal* region of the pyloric cæcum of the same fish, showing the very prominent "cæcal villi".

Fig. 3. Photomicrograph of the transverse section of the *distal* region of the pyloric cæcum of the same fish, showing the tremendous interdigitation and fusion of the "cæcal villi" to form a sort of "spongy tissue".

Fig. 4. Camera lucida sketch of the transverse section of the *distal* region of the pyloric cæcum of *Ophicephalus gachua*, showing very simple arrangement of the "cæcal villi".

Fig. 5. Photomicrograph of the transverse section of the *distal* region of the pyloric cæcum of *Notopterus notopterus*, showing the fusion of the "cæcal villi" and the formation of the intercommunicating channels.

Fig. 6. Ditto of *Mastacembelus armatus*, showing the compact gland-like arrangement of the "cæcal villi".

EXPLANATION OF THE ABBREVIATIONS USED IN THE TEXT-FIGURES AND THE PLATE

Abd. cav., Abdominal cavity; *Ant. lp.*, Anterior loop; *Bl. cap.*, Blood capillaries; *Bl. dc.*, Bile duct; *Cæ.*, Cæcum; *Cæ. lum.*, Cæcal lumen; *Cæ. pr.*, Proliferating "villi" of the cæcum; *Cæ. v.*, The so-called cæcal villi; *Ch.*, Inter-communicating channels inside the cæcal lumen; *Circ. musc.*, Layer of circular muscle fibres; *D. Cæ.*, Dorsal cæcum; *F. tis.*, Adipose tissue; *Gl. bl.*, Gall-bladder; *Int.*, Intestine; *Int. dig. muc. fl.*, Interdigitation and fusion of the mucous folds of the cæcum; *Int. vil.*, the so-called intestinal villi; *L. Cæ.*, Left cæcum; *Liv.*, Liver; *Long. musc.*, Layer of longitudinal muscle fibres; *Mus.*, Muscles; *Oes.*, Oesophagus; *Ov.*, Ovary; *Post. lp.*, Posterior loop; *Pyl.*, Pylorus; *R. Cæ.*, Right cæcum; *Ser.*, Serosa; *St.*, Stomach; *Sub. muco.*, Sub-mucosa; *V. Cæ.*, Ventral cæcum.

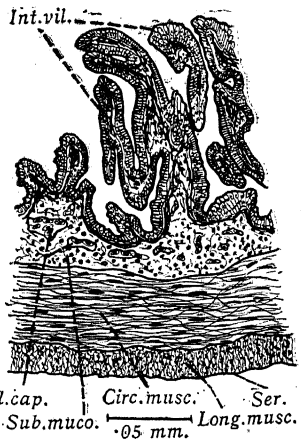


FIG. 1

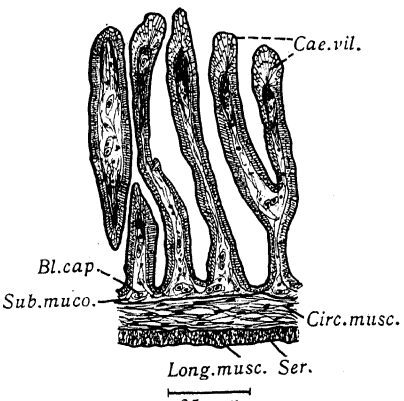


FIG. 2

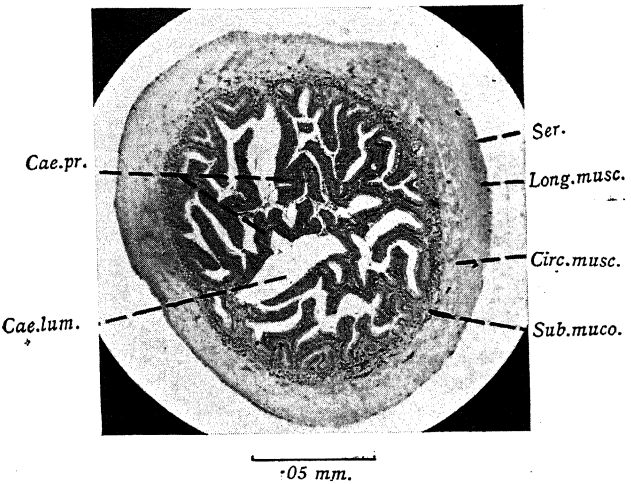


FIG. 3

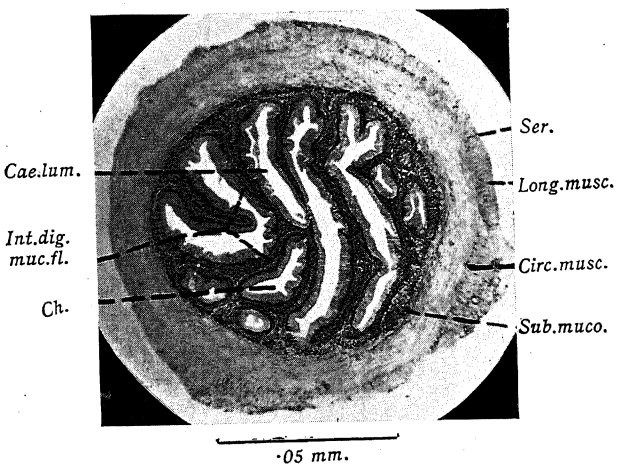


FIG. 5

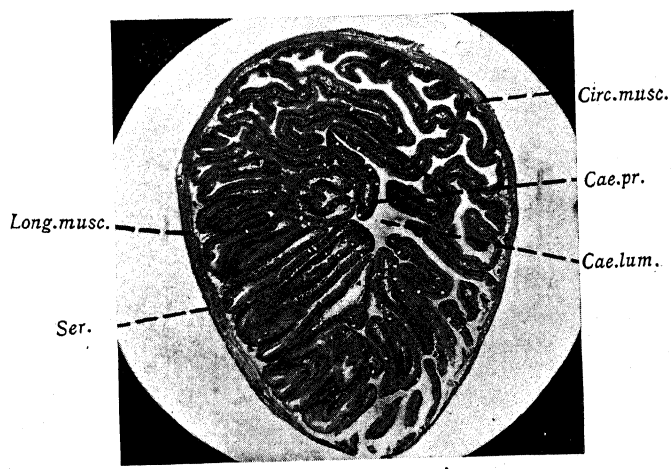


FIG. 4

STUDIES ON THE HELMINTH PARASITES OF KASHMIR*

Part II. On Two New Trematodes of the Subfamily *Pleurogenetinae* Looss (1899) with a Review of the Genus *Pleurogenes* Looss (1896)

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[Communicated by Dr. G. S. Thapar, M.SC., PH.D. (London), F.A.SC.]

THE trematode parasites, described in the present communication, form a part of the author's collection, from 1938–1941, from Kashmir. The entire work on these forms was done in the Zoological Laboratories at Lucknow under the supervision and guidance of Dr. G. S. Thapar to whom the author is indebted for his valuable suggestions and helpful criticism during the course of the work, and for keeping at the disposal of the author his personal library which is full of valuable literature. The author also wishes to thank Dr. S. L. Hora, Director of Fisheries, Bengal, for the identification of the hosts and Dr. M. B. Lal for advice and revision of the manuscript.

Prosotocus kashabia (n.sp.)

Earlier reference to Indian species of this genus is that by Mehra and Negi (1928), who described *P. indicus* from the intestine of *Rana tigrina*. Srivastava (1933) gave an account of *P. infrequentum* and Pande (1937) of *P. himalayai*, both from *Rana cyanophlyctis*. The present communication adds another species to the genus obtained from the intestine of *Rana cyanophlyctis* at Srinagar, Kashmir.

Description.—Body is more or less oval and spinose bearing exceedingly small spines arranged in regular rows.† Length of the body varies from 0·75 to 1·44 mm. and the maximum breadth is 0·38–0·66 mm. Oral sucker is subterminal, more or less oval, broader than long and 0·075–0·15 × 0·1–0·17 mm. in size, with mouth facing ventrally. Ventral sucker is equatorial or slightly post-equatorial, circular, 0·1–0.13 mm. in diameter and lies at a distance of 0·35–0. 67 mm. from the anterior end.

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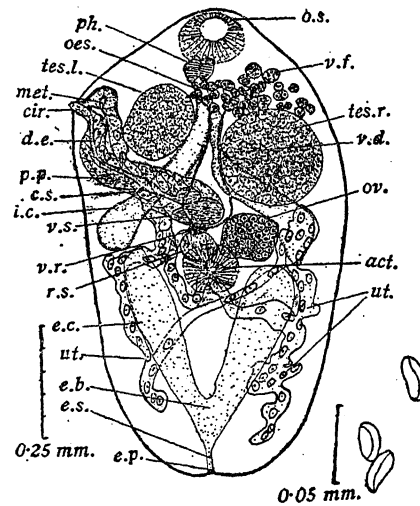
* For Part I of the "Studies on the Helminth Parasites of Kashmir," *vide Proc. Ind. Acad. Sci.*, 1941, **13**, 369–78.

† Spines are not shown in the figure.

Pharynx is globular, 0·03–0·06 × 0·05–0·07 mm. in size and œsophagus is longer than pharynx and measures 0·09–0·23 × 0·02–0·04 mm. Intestinal bifurcation is situated in the anterior quarter of the body. Intestinal cæca do not extend beyond the posterior limits of acetabulum, and its blind ends are slightly post-equatorial.



A (1) A (2)

Fig. A (1) *Prosotocus kashabia* (n.sp.) from *Rana cyanophlyctis* (dorsal view).
Fig. A (2) *Prosotocus kashabia*, eggs.
(*N.B.*—In all the figures spines have not been shown.)

Testes are pre-acetabular, and lie at the level of intestinal fork, extracæcal or overlapping the intestinal cæca in front of ovary. They are spherical, slightly oblique and unequal, the left one 0·13 × 0·12—0·17 × 0·15 mm. and the right one 0·15 × 0·12—0·22 × 0·22 mm. Cirrus sac is pre-acetabular, 0·3—0·43 × 0·08—0·11 mm. in size, and lies at a distance of 0·02—0·06 mm. from it. Its proximal basal portion is swollen and oblique and the distal tubular portion is more or less straight and lies near left margin. Vesicula seminalis lies inside the basal swollen portion of cirrus sac and consists of two parts, *i.e.*, the proximal broad sac-like portion and distal coiled tubular portion. Pars prostatica is flask-shaped measuring 0·06 × 0·03 mm. and is enclosed by prostate gland cells. Ductus ejaculatorius and cirrus are present in the distal half of cirrus sac, near lateral margin.

Ovary is somewhat pear-shaped and is post-testicular but pre-acetabular in position, being slightly on the right side. It lies dorsal to cæcum and is smaller than both the testes, measuring 0·06 × 0·09—0·11 × 0·14 mm. in

size. Receptaculum seminis varies in size and position. It may be pre-acetabular or acetabular and is spherical or oval 0·02 × 0·05 —0·05 × 0·09 mm. in size. Vitellaria bear vitelline follicles, all aggregated towards the right side of œsophagus between it and the right margin. The follicles overlap œsophagus and also anterior part of right testis. Two vitelline ducts are present which fuse to form a small triangular vitelline-vesicle near the receptaculum seminis. Owing to the asymmetry in the position of vitellaria, both the vitelline ducts arise from the same side. Uterus presents a few loops and is both pre- and post-acetabular, but never extends in front of intestinal fork. Metraterm is present but not very well differentiated. Genital atrium is indistinct and genital pore is sinistral, pre-acetabular and nearly marginal, lying on a level in front of intestinal bifurcation. Eggs are oval and measure 25—30 × 12 μ.

Excretory bladder Y-shaped with a small median stem, 0·06 mm. long and the lateral cornua are slightly inflated (0·1—0·11 mm. broad) and extend in the acetabular region, upto the blind ends of intestinal cæca. Excretory pore is terminal.

Discussion.—The present form differs from *P. fuelleborni* in the position of genital pore, which in the latter case is post-testicular and lies at a level posterior to the intestinal fork. It also differs from *P. confusum* and *P. fuelleborni* in the position of ovary and cirrus sac, for in the latter two species, ovary lies median and cirrus sac extends not only in acetabular and pre-acetabular regions but also in the post-acetabular region. It resembles *P. indicus* and *P. infrequentum* in the shape and position of gonads but can readily be distinguished from them by the peculiar one-sided position of vitellaria. All the vitelline follicles are confined in the present species towards the right side of œsophagus and in this character it resembles *P. himalayai.* These two species differ from each other in the size of body, the length of œsophagus, the posterior extension of intestinal cæca, the position of receptaculum seminis and shell gland mass, the position, shape and size of gonads and the extent of uterine loops. *P. himalayai* has body, and œsophagus proportionately long, intestinal, cæca extend a little beyond acetabulum, receptaculum seminis and shell gland mass is post-acetabular and ovary is extracæcal and larger than the testes. In the present form, on the other hand, the length of body is about twice that of its breadth, œsophagus lies in the anterior fourth of the body, intestinal cæca do not extend beyond the level of acetabulum, receptaculum seminis and shell gland mass is acetabular or pre-acetabular, ovary is smaller than the testes and never entirely extracæcal and uterine loops are fewer than that of *P. himalayai.* It is therefore described as a new species *P. kashabia.*

Pande (1937) reports the presence of only one vitelline duct in his species *P. himalayai* and on the basis of this observation presumes the existence of only one vitelline gland. *P. kashabia* is similar to *P. himalayi* in the distribution of vitelline follicles and the author has observed two well-marked vitelline ducts in it. Moreover the one-sided position of vitellaria is also present in certain species of *Pleurogenoides*, for example in *Pleurogenoides sphericus* Klein (1905) and *Pleurogenoides taylori* Tubangui (1928). In the former, two vitelline ducts have been traced to arise from the right side where all the vitelline follicles appear to have aggregated. The author believes that both the vitelline glands are present in those species of *Prosotocus* and *Pleurogenoides* which show this peculiar one-sided distribution of vitelline follicles. It appears that owing to the enlargement and anterior extension of cirrus sac and possibly in order to facilitate its movements and that of metraterm during the act of copulation the vitelline follicles of the left side have shifted towards the right side of œsophagus. In the light of above observations, regarding the number of vitelline ducts and vitelline glands, the species *P. himalayai* needs re-examination.

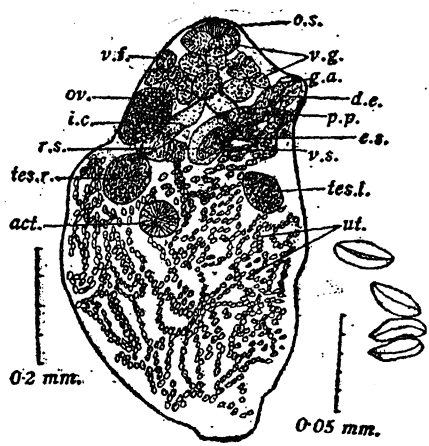
Key.—

1.	Genital pore post-testicular	*P. fuelleborni.*
	Genital pore testicular or pre-testicular ..	2
2.	Ovary median; cirrus sac post-acetabular to pre-acetabular	*P. confusum.*
	Ovary lateral; cirrus sac pre-acetabular ..	3
3.	Vitellaria on both sides	4
	Vitellaria confined on one side only ..	5
4.	Genital pore in the level of testes; ovary and testes more or less equal in size ..	*P. indicus.*
	Genital pore pre-testicular; ovary much smaller than testes	*P. infrequentum.*
5.	Ovary larger than testes; intestinal cæca extend a little beyond acetabulum ..	*P. himalayai.*
	Ovary smaller than both testes; intestinal cæca do not extend beyond acetabulum ..	*P. kashabia* (n.sp.)

Pleurogenoides Bufonis (n.sp.)

Travassos (1921) created the genus *Pleurogenoides* to include such species of *Pleurogenes* as have short intestinal cæca, never extending beyond acetabulum. *Pleurogenoides* comprises thirteen species and are chiefly parasites of Amphibia. *P. tener, P. minus* and *P. pabdai* are exceptional, the former is a reptilian parasite reported from *Chamæleo basiliscus* and the latter two

are piscine trematodes found in the intestine of *Esox lucius* and *Callichrus pabda* respectively. The genus has a wide distribution being reported from all continents. There are four Indian species:—*P. gastroporus* Lühe (1901) and *P. sitapurii* Srivastava (1934) are reported from *Rana cyanophlyctis*,



B (1) B (2)

Fig. B (1) *Pleurogenoides bufonis* (n.sp.) from *Bufo viridis* (ventral view).

Fig. B (2) *Pleurogenoides bufonis*, eggs.

P. sphericus Klein (1905) from *Rana hexadactyla* and *P. pabdai* Pande (1937) from the fish *Callichrus pabdai*. Mehra and Negi (1928) added *P. gastroporus* var. *equalis*, a new variety, from *Rana tigrina*, but Bhalerao (1936) fused the variety with *P. gastroporus* Lühe (1901). The present communication adds another species collected from the intestine of the toad, *Bufo viridis*, in August 1940, at Srinagar, Kashmir. The species appears rare, for only one out of thirty-five toads examined, was infected with this parasite.

Description.—Body is delicate, thin and transparent. It is oval in form, measuring 0·73—0·82 mm. in length and 0·42—0·45 mm. in maximum breadth and bears large number of eggs in the posterior two-third of the body. The anterior part is studded with rows of minute spines which alternate in the adjacent rows and disappear beyond the level of ovary. Suckers are feebly muscular and owing to the presence of large vitelline follicles and brown ova, their outline can be traced with difficulty. Oral sucker is subterminal and oval, measuring 0·08—0·1 × 0·05—0·06 mm. Acetabulum is equatorial or slightly pre-equatorial, smaller than oral sucker and circular in outline, with 0·06—0·07 mm. in diameter. Excretory pore is terminal.

Pharynx is present, measuring 0·022 × 0·014 mm. and œsophagus is moderately long, 0·044 mm. in length. Intestinal cæca are short, subequal divergent, slightly dilated, pre-equatorial, pre-acetabular and pre-testicular.

Testes are round to oval, symmetrically placed, and pre-acetabular in position. They are subequal; the right testis is 0·09—0·12 × 0·06—0·07 mm and the left testis 0·08—0·1 × 0·06—0·08 mm. Cirrus sac is well developed, sac-like and 0·23 × 0·06 mm. in size. It is sinistral, lies on the ventral side of the left intestinal cæcum and extends obliquely from the level immediately in front of testes to the genital pore. Vesicula seminalis is coiled and is divided into basal sac-like portion, 0·11—0·12 × 0·035—0·04 mm. in size and the distal tubular region. Pars prostatica is globular to flask shaped, measuring 0·035—0·055 × 0·025 and is followed by ductus ejaculatorius and cirrus.

Ovary is oval, 0·12 × 0·07—0·08 mm. in size. It is extracæcal and pre-testicular and lies between the vitelline follicles and the right testis Receptaculum seminis is 0·052 × 0·048 mm. in size and lies dorsal to the right intestinal cæcum between ovary and right testis. A diffused shell gland mass is present just near the receptaculum seminis on its inner side. Vitellaria extend dorsally in the entire anterior region of the body upto the level of ovary, intestinal fork and the distal portion of cirrus sac. There are 9–18 vitelline follicles of large size circular to oval, measuring 0·02 × 0·02—0·05 × 0·04 mm. They are so conspicuous that they mask the outlines of oral sucker, pharynx and œsophagus. Uterus is well developed and occupies about the posterior two-third of the body. As the parasites are all mature, the uterine coils are indistinguishable. Metraterm lies dorsal to cirrus sac and opens into the genital atrium which lies in the slight protuberance, present on the left body margin at the level of the intestinal fork and contains both male and female openings. It communicates outside through the genital pore which lies on the left body margin at the level of intestinal bifurcation or a little in front of it. Eggs are oval and slightly elongated, 28—30 ×12—14μ in size and yellowish to dark brown in colour.

Discussion.—As will be discussed later in this paper the author agrees with Travassos (1921, 1930 and 1931) in retaining *Pleurogenoides* as a separate genus comprising thirteen species. A new distome, *Pleurogenoides bufonis* has been added in the present communication. *P. bufonis* has acetabulum equatorial or slightly pre-equatorial and thus the species differs from *P. sphericus*, *P. solus*, *P. tener* and *P. sitapurii* in all of which acetabulum is distinctly post-equatorial. In the extra-cæcal position of ovary

the new distome resembles *P. pabdai*, *P. madians*, *P. minus* and *P. japanicus* but differs from the species *P. freycineti*, *P. arcanum*, *P. taylori*, *P. stromi* and *P. gastroporus*. It can be separated from *P. pabdai* by the shape of body and intestinal cæca and relative position of acetabulum and testes. It differs from *P. minus* in the arrangement of vitelline follicles, the form of cirrus sac, the position of genital pore and the posterior extension of intestinal cæca. It is different from *P. medians* in the position of testes in relation to body and acetabulum and in the number and size of vitelline follicles. It differs from *P. japonicus* in the relative position of the matraterm to the cirrus pouch, arrangement of follicles in vitellaria and size of ovary in relation to testes. It is therefore considered as a new species under the genus.

Key.—The following key is extended and slightly modified from Srivastava (1934) :—

1.	Acetabulum distinctly post-equatorial ..	2
	Acetabulum equatorial or pre-equatorial ..	5
2.	Oesophagus present	3
	Oesophagus absent	*P. sphericus.*
3.	Intestinal cæca extend upto the acetabulam ..	*P. solus.*
	Intestinal cæca do not extend upto the acetabulum	4
4.	Excretory pore terminal	*P. tener.*
	Excretory pore subterminal	*P. sitapurii.*
5.	Ovary extracæcal	6
	Ovary not extracæcal	10
6.	Acetabulum pre-equatorial; posterior end of the body bifid	*P. pabdai.*
	Acetabulum equatorial or slightly pre-equatorial; posterior end of the body not bifid..	7
7.	Intestinal cæca extend upto the level of acetabulum	*P. minus.*
	Intestinal cæca do not extend upto the level of acetabulum	8
8.	Testes pre-equatorial; vitelline follicles few and of large size	*P. bufonis* (n.sp.).
	Testes equatorial; vitelline follicles numerous and of small size	9
9.	Intestinal cæca reach to the level of the testes	*P. japanicus.*
	Intestinal cæca do not reach to the level of the testes	*P. medians.*

10.	Oesophagus present	11
	Oesophagus absent	12
11.	Genital atrium opens on the left body margin	*P. freycineti.*
	Genital atrium opens subterminally halfway between the left intestinal cæcum and body margin	*P. arcanum.*
12.	Testes situated anterior to the ends of the intestinal cæca	*P. taylori.*
	Testes situated behind the ends of the intestinal cæca	13
13.	Vitellaria consists of a few large follicles, precæcal	*P. stromi.*
	Vitellaria consists of a large number of small follicles scattered all over the cæca and meeting in the median line	*P. gastroporus.*

Review of the Genus Pleurogenes *Looss* (1896)

Pleurogenes Looss (1896) forms a composite group of heterogeneous species and Travassos (1921 and 1928) divided it into two genera—*Pleurogenes* and *Pleurogenoides*—the former with elongated intestinal cæca extending posteriorly beyond acetabulum and the latter with short cæca which do not extend beyond acetabulum. Travassos restricted the genus *Pleurogenes* to such species as *P. claviger*, *P. loossi* and *P. lobatus* and all the remaining species were placed by him under the new genus *Pleurogenoides*. Mehra and Negi (1928) gave them only sub-generic status and called the two genera *Pleurogenoides* and *Pleurogenes* as *Pleurogenes* (*Pleurogenes*) and *Pleurogenes* (*Telogonella*). Srivastava (1934) supported Mehra and Negi but Macy (1936) retained *Pleurogenes* and *Pleurogenoides* as separate genera in his key on Pleurogenetinæ. The author believes that the view expressed by Mehra and Negi and later supported by Srivastava is not correct. *Pleurogenoides* and *Pleurogenes* differ not only in the length of intestinal cæca but also in the position of testes which is always acetabular or pre-acetabular in the former and distinctly post-acetabular in the latter. *Pleurogenes orientalis* definitely resembles in both these characters to the species of *Pleurogenes* Travassos (1921) and can be safely placed under it. These differences between *Pleurogenes* and *Pleurogenoides* are no less important and significant than what we notice between *Mehraorchis* (an unfortunate nomenclature) and *Prosotocus* and hence they must be given separate generic status.

Stafford (1904) created the genus *Loxogenes*, for *Pleurogenes arcanum* (syn. *Distomum arcanum* Nickerson, 1900), on account of the genital pore being present on the ventral surface, midway between the left intestinal cæcum and the body margin. Klein (1907), Mehra and Negi (1928) and Srivastava (1934) do not subscribe to this view and have placed *Loxogenes arcanum* under *Pleurogenes*, but Tubangui (1928), Fuhrmann (1928) have retained *Loxogenes* as a separate genus. Krull (1933) added one more species to the genus from *Rana clamitans* and called it *Loxogenes bicolor*.

Loxogenes arcanum Stafford (1904) and *Loxogenes bicolor* Krull (1933) possess more points of difference than resemblance. Apart from the difference in the length of cæca, the distribution of vitelline follicles and the form of excretory bladder, they differ in the topography of gonads as well as in the position of cirrus sac. *L. arcanum* has (*a*) short cæca, (*b*) vitelline follicles present across entire body from pharynx to acetabulum, (*c*) excretory bladder with short stem and inflated cornua, (*d*) ovary pre-acetabular between two testes and (*e*) cirrus sac sinistral. *L. bicolor* has (*a*) elongated inflated cæca, (*b*) vitellaria mainly confined laterally, (*c*) excretory bladder with long stem and small cornua, (*d*) ovary acetabular and testes post-acetabular and (*e*) cirrus sac dextral. Thus the two species cannot be retained under one and the same genus. As the position of genital pore which is the most important generic character is disputed in *L. arcanum* (on ventral surface according to Nickerson, 1900 and Stafford, 1904; on dorsal surface behind pharynx and near intestinal fork according to Osborn, 1912), the exact systematic position of the species becomes difficult. In view of its resemblance with *Pleurogenoides* in the topography of gonads, form of excretory bladder, sinistral position of cirrus sac and extent of intestinal cæca it may be tentatively named *Pleurogenoides arcanum*.

The description of the adult worm of *Loxogenes liberum* Senso (1907) being in Japanese script is not available and its metacercariæ described by Yamaguti (1937) shows difference from *L. bicolor* in the shape of excretory bladder and sinistral arrangement of cirrus sac. As the form of excretory bladder remains constant during development and difference in this character is of generic importance the two species, *L. bicolor* and *L. liberum*, cannot be placed in one and the same genus.

Loxogenes bicolor differs from *Pleurogenes* and *Pleurogenoides* in (*a*) long inflated intestinal cæca, (*b*) shape of excretory bladder, (*c*) dextral position of cirrus sac and genital opening, (*d*) median and acetabular position of ovary and (*e*) folded condition of testes. Thus the author agrees with Krull (1933) and Macy (1936) in retaining *Loxogenes* as a separate genus and further thinks that *Loxogenes bicolor* is its sole representative.

Loxogenes *Krull* (1933) *Emended*

Generic diagnoses.—Medium sized distomes with broad flattened and thick bodies; cuticle spinous; oral sucker subterminal and well developed; acetabulum small, weakly muscular and slightly pre-equatorial; prepharynx absent; pharynx present; œsophagus short and slender; intestinal cæca long, reaching near the posterior end of body and inflated; testes large, elongated dorsoventrally and folded, post-equatorial and opposite each other; cirrus sac long and slender, pre-equatorial and dextral; ovary rounded, lobed, or irregular in shape and pre-testicular, lying dorsal to acetabulum; receptaculum seminis and Laurer's canal present; shell glands well developed; vitellaria follicular, preacetabular with greatest concentration laterally; uterus voluminous both pre- and post-acetabular; metraterm short and well differentiated; genital pore ventral and pre-acetabular lying slighty right of median line; excretory vesicle Y-shaped with a long stem and short cornua; excretory pore posterior and terminal; parasites of Amphibia.

Type Species Loxogenes bicolor

Ozaki (1926) described a fluke from the bile ducts of the Japanese frog, *Polypedates buergeri* and placed it under *Pleurogenes* as *P. lobatus.* This worm shows the following differences from *Pleurogenes* :—(1) genital pore is situated at a level posterior to intestinal bifurcation, (2) Ovary is median and acetabular in position, (3) gonads (both ovary and testes) are greatly lobed and (4) vitelline follicles are cæcal and arranged in a number of small bunches.

Owing to its difference from *Pleurogenes,* in the position of genital pore and ovary and arrangement of vitellaria, this worm cannot be included in the genus. The author therefore creates a new genus *Pleurolobatus* for the reception of *Pleurogenes lobatus.*

Pleurolobatus (*n.g.*)

Generic diagnosis.—Distomes of moderate size; body oval and spinose ; oral sucker subterminal; acetabulum smaller than oral sucker and pre-equatorial ; pharynx and prepharynx present; œsophagus short; intestinal cæca long extending to the last quarter of body length; testes large, irregularly lobed, equatorial and post-acetabular lying ventral to cæca; cirrus sac long and conical, pre-acetabular and more or less transversely placed; ovary large, irregularly lobed, acetabular and slightly pre-acetabular and median; receptaculum seminis and Laurer's canal present; shell glands median and in front of ovary; vitellaria follicular in five to ten groups on each side, lying ventral to cæca between intestinal fork and

testes; uterus mostly post-testicular with more or less transverse loops; metraterm long and well differentiated; genital pore dorsal, pre-acetabular, close to left margin, behind the level of intestinal fork; excretory vesicle V-shaped; excretory pore terminal; parasites of Amphibia.

Type Species Pleurolobatus Lobatus

Thus the old complex genus *Pleurogenes* Looss (1896) is split into four genera—*Pleurogenes* as emended by Travassos (1921), *Pleurogenoides* Travassos (1921), *Loxogenes* Krull (1933) emended and *Pleurolobatus* (n.g.) and the distribution of the various species is as follows :—

1. *Pleurogenes* Looss (1896) emended by Travassos (1921) includes *P. claviger* Looss (1899), *P. intermedius* Issaitschikow (1926), *P. loossi* Travassos (1930) and *P. orientalis* Srivastava (1934).
2. *Pleurogenoides* Travassos (1921) includes *P. medians* Olsson (1876), Looss (1899), *P. tener* Looss (1899), *P. gastroporus* Lühe (1901), Mehra and Negi (1928), *P. sphericus* Klein (1905), *P. arcanum* Klein (1905), *P. freycineti* Johnson (1912), *P. solus* Johnson (1912), *P. taylori* Tubangui (1928), *P. stromi* Travassos (1930), *P. minus* Pigulewsky (1931), *P. sitapurii* Srivastava (1934), *P. japanicus* Yamaguti (1936), *P. pabdai* Pande (1937) and *P. bufei* (n.sp.).
3. *Loxogenes* Krull (1933) emended, includes *L. bicolor* Krull (1933) syn. *Pleurogenes bicolor* Srivastava (1934).
4. *Pleurolobatus* (n.g.) includes *Pleurolobatus lobatus* (syn. *Pleurogenes lobatus* Ozaki, 1926).

LIST OF REFERENCES

1. Bhalerao, G. D. .. "Studies on the Helminths of India; Trematoda III," *Jour. Helminth.*, 1936, **14**, No. 4.
2. Fuhrmann, V. O. .. "Trematoda" in Kukenthal and Krumbach's *Handbuch der Zoologie*, 1928.
3. Krull, W. H. .. "*Loxogenes bicolor*, a new pigmented fluke from the frog *Rana clamitans*," *Trans. Amer. Micros. Soc.*, 1933, **52.**
4. Looss, A. .. "Weitere Beitrage Zur Kenntnis der Trematodenfauna Aegyptens," *Zool. Jaharb. Syst.*, 1899, 12.
5. Macy, R. W. .. "A new genus and species of Trematodes from the little brown bat and a key to the genera of Pleurogenetinæ," *Proceed. U. S. Nat. Mus.*, 1936, **83.**
6. Mehra, H. R., and Negi, P. S. .. "Trematode parasites of the Pleurogenatinæ from *Rana tigrina* with a revision and synopsis of the subfamily," *Allahd. Univ. Stud.*, 1928, **4.**
7. Modlinger .. "*Prosotocus* spp.," *Stud. Zool. Budapest*, 1930, **1.** (Not available.)
8. Modlinger .. "Trematode Ungarischer Chiropterin," *Ibid.*, 1930, **1.** (Not available).

9. Pande, B. P. .. "Two new fish Trematodes from Allahabad," *Proc. Nat. Acad. Sci., Ind.*, 1937, **7**, Part 2.

10. ——— .. "*Prosotocus himalayai* (n.sp.); a frog Trematode (Lecithodendriidæ)," *Ibid.*, 1937, **7**, Part 3.

11. Pigulewsky, S. W. .. "Neue Arten von Trematoden aus Fischen des Dnjeprbassins," *Zool. Anz.*, 1931, **96**.

12. Srivastava, H. D. .. "On new Trematodes of frog and fishes of the United Provinces, India; Part II. Three new trematodes of the subfamily Pleurogenetineæ (Fam. lecithodendriidæ) from *Rana cyanophlyctis* of Oudh," *Bull. Acad. Sc. U.P., Allahabad*, 1939, **4**, No. 4.

13. ——— .. "On new Trematodes of frog and fishes of the United Provinces, India; Part III. On a new genus *Mehraorchis* and species of *Pleurogenes* (Pleurogenetinæ) with a systematic discussion, and revision of the family Lecithodendrūdæ," *Ibid.*, 1934, **4**, No. 4.

14. Travassos, L. .. "Contribuicoes Para O Conhecimento Da Fauna *Helmnthologica brasileira*, XV, Sobre as especies Brasileiras da Fauna Lecitho dendriidæ Assisteule do instituto Oswald Cruz," *Arch. de. Esc. Sup. de Agric. e. Med. Veter., Nictheroy*, 1921, **5**, Nos.1, e.2.

15. ——— .. "Pesquizas Helminthologicus realisadas em Hamburgo, V, Genero *Prosotocus* Looss, 1899 (Trematoda; Lecithodendriidæ)," *Mem. Inst. Osw. Cruz.*, 1930, Tomo. 24, Fasc. 2.

16. ——— .. "*Ibid.*, VI, Genero *Pleurogenoides* Travassos 1921 (Trematoda; Lecithodendriidæ)," *Ibid.*, 1930, Tomo. 24, Fasc. 2.

17. ——— .. "*Ibid.*, VIII, Informacœ Sobre O genero *Pleurogenes* Looss 1896, (Trematoda; Lecithodendriidæ)," *Ibid.*, 1930, Tomo. 24, Fasc. 4.

18. Tubangui, M. A. .. "Trematode parasites of Philippine Vertebrates," *Phil. Journ. Sci.*, 1928, **36**, No. 3.

19. Ward, H. B., and Whipple, G. C. .. '*Fish-water Biology*,' 1918.

20. Yamaguti, S. .. "Studies on the Helminth Fauna of Japan; Part 14, amphibian Trematodes," *Jap. Jour. Zool.*, 1934–36, **6**.

21. ——— .. "Second intermediate host of *Loxogenes liberum*," *Jour. Parasit.*, 1937, **23**.

LETTERING

act., acetabulum; *cir.*, cirrus; *c.s.*, cirrus sac; *d.e.*, ductus ejaculatorius; *eg.*, egg, *e.b.*, excretory bladder; *e.c.*, excretory cornua; *e.p.*, excretory pore; *e.s.*, excretory bladder, median stem; *g.a.*, genital atrium; *g.p.*, genital pore; *i.b.*, intestinal bifurcation; *i.c.*, intestinal cæcum; *mo.*, mouth; *met.*, metraterm; *æs.*, œsophagus; *o.s.*, oral sucker; *ov.*, ovary; *ovd.*, oviduct; *ph.*, pharynx; *prp.*, prepharynx; *p.p.*, pars prostatica; *r.s.*, receptaculum seminis; *s.g.*, shell gland; *tes.*, testis; *tes.r.*, right testis; *tes.l.*, left testis; *ut.*, uterus; *v.d.*, vitelline ducts; *v.f.* vitelline follicles; *v.g.*, vitelline gland; *v.r.*, vitelline reservoir; *v.s.*, vescicula seminalis.

(Type specimens and cotypes are deposited in Dr. Thapar's Helminthological Collections, Lucknow University).

ROOT INITIATION IN THE ADULT AXES OF A FEW DICOTYLEDONOUS SPECIES

(With 14 Text-Figures)

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Introduction

ORIGIN of shoot borne adventive roots is described in text-books as *endogenous*, exceptions being recorded in the family of Crucifereæ, *e.g.*, in *Cardamine pratensis* (Hansen, 1881) and *Nasturtium austriacum* (= *Roripa austriaca*, Wilson, 1927). In both these cases the origin is described as *exogenous* and is reported to be associated with axillary buds (Priestley and Swingle, 1929).

In assigning the position of the endogenous root initials in an organ De Bary (1884) makes the general statement that it must be " in or close to vascular bundles or masses of wood or bast " (p. 315). Lemaire (1886) made extensive studies of the " origin of naturally occurring adventive roots in the hypocotyls, stolons and rhizomes " of herbaceous dicotyledons and observed that the origin of these roots might be (1) exclusively in the pericycle, (2) partly in the pericycle and partly in the endodermis and inner cortex, (3) exclusively in the " subphloem meristem ", *i.e.*, cambium, and (4) partly in the cambium and partly in the pericycle. He, however, concluded that the pericyclic origin is by far the commonest. Van Tieghem and Douliot (1888) fix the place of origin entirely in the pericycle so far as the young hypocotyls, epicotyls, stolons and rhizomes are concerned; if, however, they state, the pericycle loses its " root-forming property ", as in old stems, the adventive roots arise in the phloem parenchyma, both primary and secondary; and still later in cambium itself. Eames and MacDaniels (1925), on the other hand, give the position as the pericycle in the cases of young stems and in older axes, where the pericycle is no longer active, in the secondary phloem (p. 238).

Priestley and Swingle (1929) distinguished the shoot borne adventive roots into two categories on the basis of their origin, namely, (1) those formed behind the apical meristem and (2) those arising upon old stems which have

ceased to extend longitudinally, but in which radial growth alone is proceeding (p. 62). They then pointed out that in young stems these initials differentiate practically always near but to the side of a vascular group, *i.e.*, on a primary ray. In old stems on the other hand the site of initiation of a lateral root moves inwards from the region of the pericycle to the living cells of the ray that lie close to the newly differentiated xylem and phloem. The root initials are formed in association with a group of cells bordering upon the vascular cambium (pp. 63, 64).

We have undertaken this investigation in order to determine in a general way the origin of naturally occurring adventive roots on adult axes of herbaceous plants, and at the same time to re-examine the validity of the general statement of Priestley and Swingle with regard to the root initiation in old stems.

By "adult" or "old" axes we would mean the hypocotyl and internodes which have ceased to elongate longitudinally, but in which the radial extension is still going on, as contemplated by Priestley and Swingle in their second category of shoots bearing adventive roots.

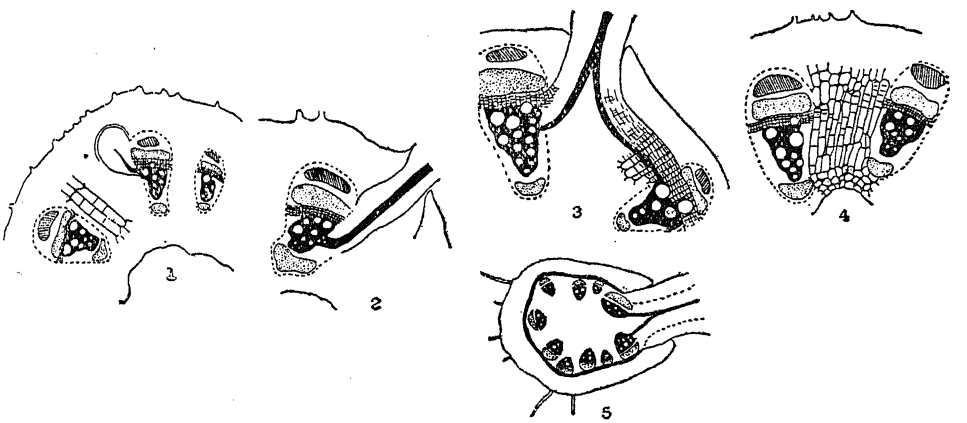
There is much controversy with regard to the use of the term 'pericycle' (Solereder, 1908; Strasburger, 1930). In this and subsequent papers we accept the definition of Van Tieghem and Douliot which runs as follows: The pericycle is "the layer or layers between the endodermis and the external phloem of a fibrovascular bundle continuous across the medullary rays but not distinguished on the inside from the medullary ray" (Carlson, p. 119, 1929).

We propose to report on the origin of naturally occurring lateral roots in the adult internodes of herbaceous dicotyledons, monocotyledons and of pteridophytes, and the present paper forms the first of the series that are expected to follow with the progress of our work. The results embodied in this paper are based on a study of free-hand sections of the hypocotyls or internodes of the species reported, and the text-figures have all been drawn under a microprojector.

Observation

Cucurbita maxima (Text-Figs. 1–4) develops a fairly elongated hypocotyl during its seedling stage of growth. The vascular bundles, ten in number, are typically bicollateral and are arranged in a ring with sclerenchymatous bundle caps characteristically associated with each of these ten bundles. These caps are very prominent in bundles near the base of the hypocotyl, but as one proceeds towards the top the cells of the cap lose their sclerenchymatous nature and do no longer give lignin reaction with aniline sulphate

or aniline chloride solution. The outer vascular cambium is much wider than the inner one, and extends laterally into a layer or two of the ray cells on the sides of each bicollateral bundle.



TEXT-FIGS. 1–5. Origin of shoot-borne lateral roots in *Cucurbita maxima* and *Oxalis corniculata*.—Figs. 1–4. *Cucurbita maxima*. T.S. of hypocotyl showing the origin of adventive roots. Fig. 4 shows the nature of the radial growth of the hypocotyl. Fig. 5. T.S. of the adult internode of *Oxalis corniculata*. ×15.

The vascular bundles in the hypocotyl thus differ from those in the stem in their arrangement round the pith, and in having isolated sclerenchymatous bundle-caps of procambial origin instead of a continuous sclerenchymatous cylinder of the adult internodes described as pericyclic in position.

Lateral roots begin to appear near the basal region of the hypocotyl which has already ceased to elongate, and proceeds to a certain distance up the organ. The root initials take their origin in the flanks of the outer cambium in the formation of which the adjoining ray cells also take part (Figs. 1 and 2). There may be two adventitious roots originating from the two sides of a vascular bundle or rarely two groups of initials from the flanks of the adjoining bundles join together, sometimes in the middle, sometimes to one side, of the intervening ray to form a single lateral root (Fig. 3). The root initials after their organization into the primordium bend through about 90° in their passage through the cortex towards the periphery of the hypocotyl without causing any or causing a very slight disturbance to the bundle-cap which retains its original position with reference to the bundle.

When the lateral roots begin to appear the hypocotyl in this region has already ceased to elongate, but the radial growth is still maintained. This is accomplished in two ways, namely, (1) by the radial growth of individual

bundles from the two fascicular cambia, particularly from the outer one, and (2) by the enormous radial elongation of ray cells which divide tangentially with a view, it appears, more to resist lateral pressure than for any other purpose (Fig. 4). Sometimes regular interfascicular cambia are organised, but they do not unite with the outer fascicular cambia to form a continuous cambial ring as in the normal axes of dicotyledons. These interfasicular cambia help the radial extension of the ray cells and also very frequently form isolated phloem patches in the rays.

Oxalis corniculata (Text-Fig. 5) is a creeping plant. Adventitious roots from adult internodes are rare in this plant. The few that have been found were given off from the lower side of the trailing shoots growing in moist shady places. There are ten bundles arranged in a ring and at no time a regular cambial ring has been seen to form though the growth and extension of the bundles, particularly of the bigger ones, is maintained by the fascicular cambia. The endodermis with casparian strips is found to persist, and a complete cylinder of thick-walled pericycle of varying depth is a special feature of the internodal structure of this plant.

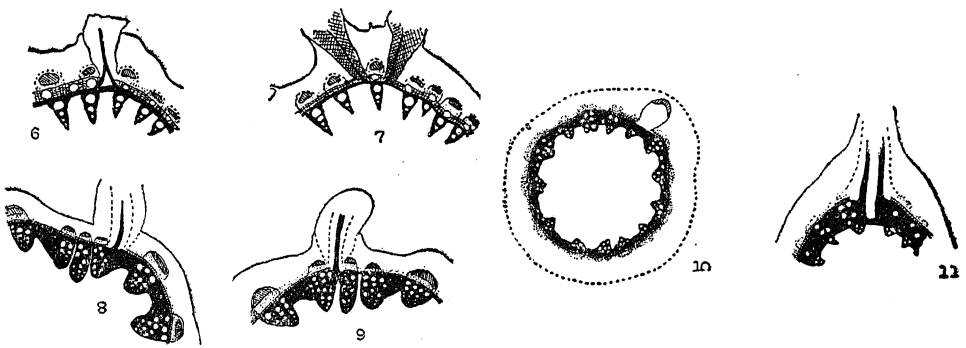
In the formation of the shoot-borne roots two groups of initials from the flanks of the adjoining vascular bundles join together in the middle of the intervening ray to form a single lateral root (Fig. 5). Vascular connection is later made with both the bundles. The root initials originate, as in *Cucurbita*, in the cambial and ray cells adjoining it.

Mikania scandens (Text-Figs. 6, 7) a twining and spreading herb which delight in moist places, particularly near the edges of a pool. Lateral roots from internodes of adult stems are very rare in this plant. The detailed developmental and adult anatomy of this plant is being worked out by Mr. I. Banerji of the Department of Botany, Calcutta University, and we propose to record here only the barest outline of the adult stem structure.

The large number of vascular bundles are surmounted each by a sclerenchymatous bundle-cap. Interfascicular cambium rarely produce any xylem vessels. In the formation of the root initials both the vascular and interfascicular cambia take part. In Fig. 6 the lateral root is median in position with reference to the medullary ray and vascular connection is made with both the bundles. In Fig. 7 two lateral roots are formed one on each side of a vascular bundle.

Tagetes patula (Text-Figs. 8, 9), an annual garden herb, but may be made to continue through many seasons if proper care is taken. It is towards the end of the first season that lateral roots, sometimes in profuse numbers, are given out from adult internodes. The origin of these roots are definitely

related to the trace bundles as their vertical arrangement can be followed along the course of these bundles in the internodes. Good varieties of *Tagetes* is propagated mainly by cuttings.



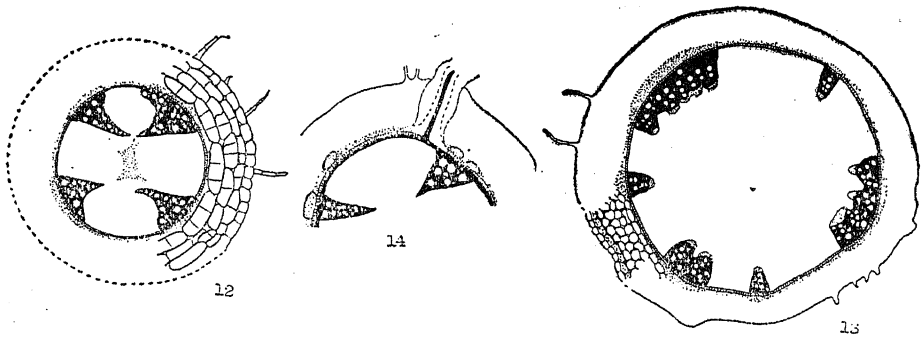
TEXT-FIGS. 6–11. Origin of shoot-borne lateral roots.—Figs. 6–7. T.S. of adult internodes of *Mikania scandens*. Figs. 8–9, that of *Tagetes patula* and Figs. 10–11, that of *Ageratum conyzoides*. ×15.

The arrangement and structure of the vascular bundles in adult internodes are typically of the *Helianthus* type. The lateral roots are given out from the flanks of the fascicular cambium of a vascular bundle. Fig. 8 shows the origin of a lateral root from the flank of a synthetic bundle in the making. The bundle-cap is not disturbed. Fig. 9 shows a root developed between two vascular bundles which has made vascular connections with both of them.

Ageratum conyzoides (Text-Figs. 10, 11).—This is also an annual herb but erect in habit. In all the specimens collected the production of lateral roots were found to be confined to the first three or four internodes growing prostrate on the ground. The anatomical structure of the axis is typically that of the woody vine type, *i.e.*, an unbroken cylinder of primary and secondary wood with no medullary rays. The lateral root arises from the flank of a vascular canbium (Fig. 10) and when it makes vascular connection with two bundles it is seen to occupy the whole of the intervening medullary ray outside the interfascicular cambium (Fig. 11).

Solanum nigrum (Text-Figs. 12–14).—This is an annual herb. Bhaduri (1933) points out that " plants identified as *S. nigrum* by Prain (1903), Hooker (1875) and others differ considerably in both morphological and cytological characters " (p. 58), and he could easily distinguish three types of *S. nigrum* from his critical studies of cytology and morphology of these plants ($n = 12$, $n = 24$, $n = 36$; pp. 60–61). We have not been able to collect, examine and compare the anatomical structure of all the three types

of Bhaduri, but Figs. 12 and 13 show that the internode and hypocotyl of this plant differ widely in their anatomical structures.



TEXT-FIGS. 12–14. Origin of lateral roots and the structure of internodes and hypocotyl in T.S.—Figs. 12 and 13. T.S. of the hypocotyl and internode respectively of *Solanum nigrum*. Fig. 14. T.S. of hypocotyl showing origin of lateral root. Figs. 12 and 14, ×15 ; Fig. 13, ×25.

Fig. 14 shows the origin of a lateral root in the hypocotyl of *S. nigrum*. It will be seen that there are only 4 primary bundles and a cambium ring is very early differentiated. The root initials originate in the interfascicular and fascicular cambia close to a vascular bundle, and the vascular connection is made with the secondary xylem and phloem.

Discussion

Adventive roots may arise on any part of a stem, but the normal position is the node. The present study as has been mentioned in the Introduction is confined to the origin of naturally occurring roots in the nodeless segments of adult shoots of a few dicotyledonous herbs ; and in this respect our observations differ from those of others dealing mostly in the regeneration of roots on stem and leaf cuttings (Swingle, 1940).

From the two reported cases it may be assumed that exogenous adventitious roots are developed only in association with axillary buds. In *Cotoneaster Dammeri* development of lateral roots in association with axillary buds is a normal feature. Miss Wolfe (1934) noted their origin in the parenchyma at the flank of the cambium of the branch (bud) trace. It is doubtful if one can call this an exogenous origin.

Lemaire and Van Tieghem and Douliot studied the origin of naturally occurring roots on hypocotyl, rhizomes and internodes, but they do not, so far as we are aware, appear to have noted any difference in the origin of lateral roots in young and adult organs. Priestley and Swingle are perhaps the first to note this difference. They formulated their second type of origin from a study of the hypocotyl in *Helianthus* and *Ricinus*, and of the

epicotyl of *Vicia* and *Solanum*. The six cases reported here are additional examples.

Trécul (1846) suggested the possibility of the existence of preformed root initials in stems of certain species. 32 years later Vöchting (1878) reported regular occurrence of preformed root premordia in the cuttings of Willow. Goebel (Vol. II, p. 275) reports the presence of pre-existing sub-cortical "root germs" in *Salix*, and these primordia are found to develop on the cuttings used for vegetative propagation in these plants. Van der Lek (1924) noticed occurrence of such preformed root germs in young branches of *Salix*, *Populus* and *Ribes nigrum* closely associated with cambium and the end of medullary rays at the outer edge of xylem. Pre-existing "root germs" have also been noticed by Swingle (1925) in apple stems and by Sandison (1934) in *Lonicera japonica*. Though *Tagetes* is often propagated by cuttings in this country we have so far failed to discover the presence of preformed root germs in this plant or in other plants examined by us. Preformed root-primordia have not also been found in *Salix caprea*, *S. aurita*, *Populus alba* and *Vitis vinifera* (Carlson, 1938).

In all the cases studied by us the site of origin is shifted to the flanks of a vascular bundle close to *cambium*, *interfascicular cambium* or *ray cells* abutting these meristematic tissues. *Cambial origin* has been reported by Corbett (1895–96) in herbaceous stem cuttings of *Geranium*, *Coleus* and allied plants; by Smith (1925, 1928) in *Coleus* and *Clematis*, by Taylor (1926) in *Acanthus montanus*, by Sandison (1934) as a response to wounding in *Lonicera japonica*, and by Arlot Smith (1936) occasionally in *Begonia maculata* and *B. semperflorens*.

Origin in *interfascicular cambium* has previously been recorded by Regal (1876) in *Begonia maculata* (= *Begonia argyrostigma*); by Connard and Zimmerman (1931) in cuttings of *Portulaca oleracea* and by A. Smith in *Begonia maculata* and *B. semperflorens*.

Pericyclic origin has been observed in *Coleus Blumei* by Carlson (1929), in *Veronica Beccabunga* by Priestley and Swingle (1929); origin in the *secondary phloem* either within or between the bundles by Carlson (1933) in the cuttings of Dorothy Perkins rose; and in the *ray parenchyma* between a poorly defined pericyclic region and the interfascicular cambium by A. Smith (1942) in the cuttings of *Tropaeolum majus*. The case reported by Crooks (1934) is very interesting in so far that in cuttings of the upper part of the hypocotyl of Flax seedlings he noticed initiation of a root by the activity of the ray cells in the regions respectively of the pericycle, phloem and pith.

According to Priestley (1931) and Priestley and Swingle (1929) three conditions must be satisfied for the initiation of a root in an organ, namely, (1) presence of a meristem or potentially meristematic cells, (2) the mother tissue must be free from air spaces and (3) placed very near the xylem and phloem. The first is necessary because by the division of its elements new growing centres of roots are organised; close proximity to vascular supply ensures adequate supply of nutritive materials and absence of air space will permit a steady diffusion of solutes (pp. 65, 66). The presence of a meristematic tissue, *i.e.*, cambium, renders grafting a practical process.

In roots the pericyclic origin of lateral roots is obviously an advantage, but in adult hypocotyl and internodes the bundles are collateral with phloem and in many cases with additional sclerenchymatous bundle-caps on the outside. Therefore to effect direct xylem connection the origin must be close to xylem in the flanks of a fascicular cambium. Intercellular spaces are already formed throughout the radial course of ray cells, the only region free from such intercellular spaces being the cambium, interfascicular cambium and the ray cells recently formed in the neighbourhood of cambial ends. The ray cells are relatively smaller and are filled with active protoplasm. When all these facts are taken into consideration the ends of the cambium, the outer region of the interfascicular cambium and the living cells just cut off from the meristematic tissue and the proximity of the newly differentiated xylem and phloem are the ideal places for the origin of a lateral root upon a radially expanding nodeless axis.

The radial growth of the hypocotyl of *Cucurbita maxima* is very interesting, but this kind of growth in thickness has already been reported in the stems of *Coccinia* (= Cephalandra), *Trichosanthes*, *Wilbrandia*, *Anisosperma* and *Alsomitra* (Potter, Schenk, Herail, in Solerder I, p. 395). In all these cases, as in the hypocotyl of *C. maxima*, the interfascicular cambia help primary rays in keeping pace with the radial expansion of the bundles.

Summary

Six additional cases of naturally occurring shoot-borne adventive roots on adult axes of herbaceous dicotyledons have been described in this paper.

These support the view of Priestley and Swingle that the site of initiation of a lateral root in adult hypocotyls and internodes moves inwards from the pericycle to the flanks of the vascular cambium close to the newly differentiated xylem and phloem.

The radial growth of the hypocotyl of *Cucurbita maxima* is maintained by the independent growth of the vascular bundles, the interfascicular

cambium helping the primary rays in keeping pace with the growth in thickness of the former.

LITERATURE

1. Bhaduri, P. N. .. "Chromose numbers of some Solanaceous plants of Bengal," *Jour. Ind. Bot. Soc.*, 1933, **12**, 56–64.
2. Borthwick, A. W. .. "The production of adventitious roots and their relation to Birds-eye formation in the wood of various trees," *Notes Roy. Bot. Gard.*, 1905, Edin. **4**, 15–36.
3. Carlson, M. C. .. "Origin of adventitious roots in *Coleus* cuttings," *Bot. Gaz.*, 1929, **87**, 119–27.
4. ———— .. "Origin and development of roots in cuttings of Rose and Willow," *Contrib. Boyce Thompson Inst.*, 1933, **5**, 313–30.
5. ———— .. "The formation of nodal adventitious roots in *Salix cordata*," *Amer. Jour. Bot.*, 1938, **25**, 721–25.
6. Clark, W. A. .. "Vegetative propagation in Cotoneaster," *Trans. & Proc. Bot. Soc.*, Edin. 1932, **31** (2), 256–61.
7. Connard, Mary H., and P. W. Zimmerman "The origin of adventitious roots in cuttings of *Portuloca oleracea* L.," *Contrib., Boyce Thompson Inst.* 1931, **3**, 337–46.
8. Corbett, L. C. .. "The development of roots from cuttings," *W. Va. Agric. Expt. Stn., Ann. Rep.*, 1895–96, **9**, 196–99 (A. I. Smith, 1936).
9. Crooks, D. M. .. "Histological and Regeneration studies on the Flax seedlings," *Bot. Gaz.*, 1934, **95**, 209–39.
10. Eames and MacDaniels .. *Introduction to Plant Anatomy*, 1925.
11. Goebel, K. .. *Organography of Plants*, Part II, 1905, p. 274, Eng. Ed. (quotes Van Tieghem and Douliot).
12. Jimenez, P. G. .. "Callus and root-formation in stem cuttings of Kapok, Achuete and Santol," *Philippine Agr.*, 1937, **26**, 585–635.
13. Van der Lek, H. A. A. .. *Over die wortelvorming van houtige stekken*, H. Veeman and Zonen, Wageningen, 1924–1925 (Carlson, 1929).
14. Lemaire, A. .. "Recherches sur l'origine et le development des racines laterales Chez les Dicotyledones," *Ann. Sci. Nat. Bot.*, Ser. VII, 1886, **3**, 163–274 (Carlson, 1929).
15. Prain, D. .. *Bengal Plants*, 2 vols., 1903.
16. Priestley, J. H. .. "Vegetative propagation from the standpoint of Plant Anatomy," Reprint, *Proc. 9th Int. Hort. Congress*, 1931.
17. ———— .. "Problems of Vegetative Propagation," *Jour. Roy. Hort. Soc.*, 1926, **51**, 1–16.
18. ——— and C. F. Swingle "Vegetative propagation from the standpoint of Plant Anatomy," *Technical Bulletin U.S. Dept. of Agriculture*, 1929, No. 151.
19. Regel, F. .. "Die vermehrung der Bigoniaceen aus ihren Blättern," *Jena, Zeitschr. Med. Naturwiss* (N.F. 3), 1876, **10**, 447–92 (A. I. Smith, 1936).
20. Sandison, S. .. "Rooting of cuttings of *Lonicera japonica*, a preliminary account," *New Phyt.*, 1934, **33**, 211–17.

21. Smith, A. I. .. "Adventitious roots in stem cuttings of *Begonia maculata* and *B. semperflorens*," *Amer. Jour. Bot.*, 1936, **23**, 511–15.

22. ———— .. "Adventitious roots in stem cuttings of *Tropæolum majus* L.," *ibid.*, 1942, **29**, 193–94.

23. Smith, E. P. .. "A comparative study of the stem structure of the genus *Clematis* with special reference to anatomical changes induced by vegetative propagation," *Trans. Roy. Soc.*, Edin., 1928, **55**, 643–44.

24. ———— .. "The origin of adventitious growth in *Cotcus*," *Trans. & Proc. Bot. Soc.*, Edin., 1925 (1936), **29**, 145–50.

25. Swingle, C. F. .. "Regeneration and Vegetative Propagation," *Bot. Rev.*, 1940, **6**, 301–55.

26. ———— .. "Burrknot formations in relation to the vascular system of the apple stem," *Jour. Agri. Res.*, 1925, **34**, 533–44.

27. Solereder .. *Systematic Anatomy of the Dicotyledons*, Eng. Ed., 2 vols., 1908.

28. Strasburger, E. .. *Text-Book of Botany*, Eng. Ed., 1930.

29. Taylor, G. .. "The origin of adventitious growth in *Acanthus montanus*," *Trans. Proc. Bot. Soc.* Edn., 1926, **29**, 291–96.

30. Van Tieghem, P., and H. Douliot "Recherches comparatives sur l'origine des membres endogenes dans les plantes vasculaires," *Ann. Sci. Nat. Bot.*, 1888, Sér. VII, **8**, 1–660 (Carlson, 1929).

31. Trécul, A. .. "Recherches sur l'origine des racenes," *ibid.*, 1846, Sér. III, **5**, 340–44 (Carlson, 1938).

32. Vöchting, H. .. *Über Organbildung im Pflanzenreich*, Theil 1, Bonn. (Carlson, 1938).

33. Wilson, C. L. .. "Adventitious roots and shoots in an introduced weed," *Bull. Torr. Bot. Club*, 1927, **54**, 35–38.

34. Wolfe, F. H. .. "Origin of adventitious roots in *Cotoneaster Dammeri*," *Bot. Gaz.*, 1934, **95**, 686–94.

ON TWO TREMATODES FROM FISHES IN INDIA

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Stomachicola muræsocis Yamaguti, 1934

Twelve specimens collected by Mr. M. Rahimullah at Ennur from the stomach of the marine eel, *Muræsox cinereus* were forwarded to the writer by Professor B. K. Das. On examination, these proved to be *Stomachicola muræsocis* Yamaguti, 1934. The specimens, in most respects, conform to the description of this species given by Yamaguti (1934), but differ from it in some minor anatomical features.

The worms measure 14·3–56·5 mm. in length and their maximum breadth which is attained in the region of the receptaculum seminis or that of the ventral sucker, is 1·3–3·3 mm. Even the smallest specimen was sexually mature. The following measurements refer to a specimen measuring 49 mm. in length and 2·7 mm. in maximum breadth. The oral sucker measures 0·47 × 0·47 mm. The pharynx measures 0·36 × 0·3 mm., and the œsophagus is 0·35 mm. long. The sinuous intestinal cæca, filled with dark amorphous contents, terminate normally at the same level, at the posterior end of the body, but in some cases the two cæca terminate at slightly varying levels. The ventral sucker is large and measures 1·8 mm. in diameter. The testes measure 0·53–0·8 × 0·33–0·42 mm. and are normally symmetrical, but in some cases the right testis may be slightly anterior to the left. The oval vesicula seminalis lies immediately posterior to the ventral sucker and measures 0·72 × 0·54 mm. The pars prostatica is long and sinuous. It is surrounded by unicellular prostatic cells and unites with the terminal portion of the uterus to form the ductus hermaphroditicus. This structure is enclosed in a small hermaphroditic pouch, which measures 0·4 × 0·23 mm. The genital pore is situated slightly behind the oral sucker.

The ovary, as described by Yamaguti (1934), is approximately kidney-shaped with its concavity directed posteriorly, and lies medially behind

the level of the testes, measuring 1·22×0·4 mm. The receptaculum seminis is very large, measures 0·5×0·4 mm., and lies behind the ovary. The vitellaria are tubular, not follicular. As described by Yamaguti (1934) the right vitelline gland normally consists of three tubes, of which the hindermost is subdivided into two similar tubes. The left vitelline gland, however, consists of two tubes of which the posterior bifurcates into two similar tubes. It may thus be seen that the vitellaria consists of seven tubes in all. Certain deviations from the normal were, however, noticed. Thus in one specimen there were five tubes on the right side, and only two on the left. In another specimen there were, on the right side, five tubes passing posteriorly and two very short but stout tubes passing anteriorly and the normal number of three tubes on the left side, thus making a total of ten tubes. Yamaguti states that the uterine coils extend into the tail region for about one-fifth of its length, but this statement could not be confirmed. In the specimens under discussion, the uterine coils were found extending into the tail region from about two-ninths to one-third of its length. The eggs are numerous and small, and measure 0·0115–0·0165 × 0·008–0·009 mm. They are much smaller in size than those in the specimens described by Yamaguti.

It will be seen, from the foregoing account, that the material at the writer's disposal differs from that described by Yamaguti in respect to the œsophagus, the vitelline tubes, the position of the genital pore, the extension of the uterus into the tail region and the size of the eggs; but the degree of differentiation is not sufficiently marked to justify the creation of a new species. It may be mentioned that Yamaguti also pointed out a few variations occurring in this species.

Clinostomum indicum Bhalerao, 1940

Through the courtesy of Professor J. N. Karve an opportunity was obtained to examine a small collection of flukes obtained from the subcutaneous tissue of the fish *Notopterus notopterus*, from Poona. All the flukes were immature, but on closer examination and comparison with the allied forms, they proved to be a new species. The short description which follows is based on three entire mounts and three sectionized series.

The worms are thick, fleshy, cream coloured, and elliptical in shape, with both the extremities rounded. The anterior border and the sides are inverted, so that the worm is canoe-shaped. In preserved specimens, the cuticle is transversely striated, and beset with spines which are noticeable only in sectionized specimens. Beneath the cuticle, there is a well-developed layer of longitudinal muscle and below this a layer of circular muscle.

There are numerous dorso-ventral muscles and many large sub-cutaneous glands. The worms measure 9·5–11 mm. in length and their maximum breadth, which is attained in the region of the genital glands, is 3·5–4·7 mm. The mouth is situated subterminally at the anterior end and is surrounded by an

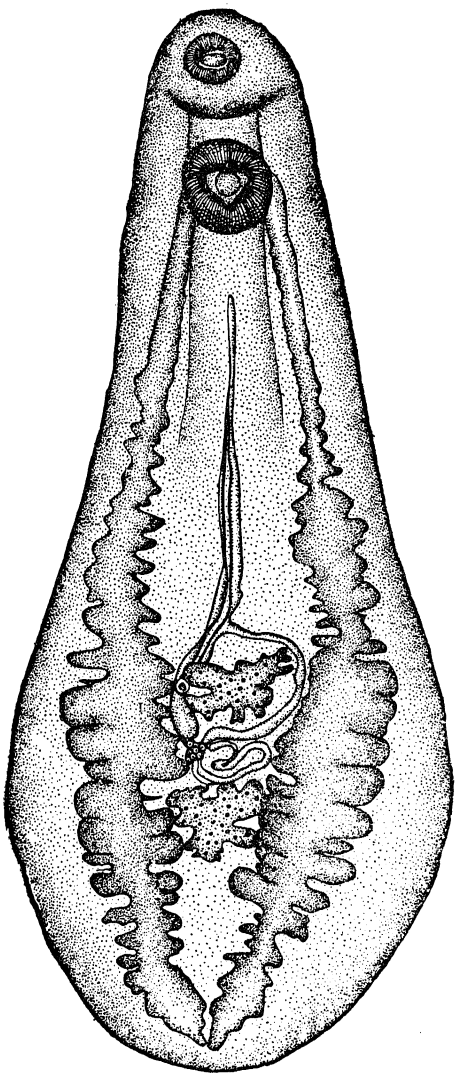

Fig. 1. Ventral view of *Clinostomum indicum* n.sp.

oral sucker measuring 0·475–0·67 × 0·375–0·57 mm. There is no pharynx. The œsophagus was observed to be short, though its actual length could not be determined on account of the peculiar bend at the anterior end of the worm. The intestinal cæca, filled with the blood of the host, pass along

the sides of the body, and terminate slightly in front of the posterior end. They are deeply diverticulated both internally and externally and some of the diverticula are subdivided into two or four digitations. The ventral sucker is very large, measures 0·86–1·18 × 0·82–0·96 mm., and is situated close to the anterior end of the body.

The excretory pore is situated subterminally at the posterior end in a small depression on the dorsal side, and leads into a V-shaped bladder with short arms. The terminations of the intestinal cæca come into close contact with the cornua of the bladder and in some cases cause an actual depression on its proximal face. In some species of this genus, it has been claimed that the intestinal cæca open posteriorly into the excretory bladder. Price (1938) while redescribing the species *Clinostomum intermedialis* Lamont (1920), remarks " intestinal cæca sinuous, apparently opening into excretory vesicle ", showing that he is sceptical about the relation existing between the intestinal cæca and the excretory bladder. Special attempts were therefore made to obtain precise information concerning this relationship in this species, these two structures being studied in two series of horizontal and in another series of dorso-ventral sections. As a result, it was ascertained that the intestinal cæca do not actually open into the excretory bladder. The two structures are intimately juxtaposed, thus giving the false impression, in entire mounts, that they directly communicate with one another. From the side of the bladder emerges a fairly prominent branch on either side, which passes anteriorly almost as far as the oral sucker.

The genital pore is situated submedianally on the right side of the anterior testis. The testes are branched, and lie centrally, one behind the other, at the middle of the posterior half of the body. The anterior testis is slightly larger than the posterior, the measurements being 1·23–1·32 × 0·67–0·8 mm. and 1·1–1·14 × 0·062–0·925 mm. The vas efferens of the anterior testis emerges from its centre and passes posterolaterally on the right side. The vas efferens of the posterior testis emerges from its centre and goes first to the right side and then turns towards the left. The two vasa efferentia unite with each other slightly behind the cirrus sac and form a very small vas deferens. Special efforts were made to determine whether there is, in this species, a vesicula seminalis externa as is described in some species, *e.g.*, in *C. piscidium* Southwell and Prashad (1918). For this purpose and for obtaining the details of the contents of the serous sac, presently to be described, a detailed study of the serial sections was made. It was ascertained that the vesicula seminalis externa is absent in this species. The cirrus sac is more or less pear-shaped, having a slight bend near its proximal end. It lies on the right side, between the anterior testis and the right

intestinal cæcum. It measures 0·8–0·84 × 0·28–0·35 mm. and extends from the genital pore to the anterior border of the ovary. The cirrus sac contains the vesciula seminalis, which is very coiled and occupies slightly more than half the space inside the cirrus sac. The vesicula seminalis is followed by the ductus ejaculatorius, which is 0·24 mm. long and 0·08 mm. thick. The terminal portion of the male genital duct is the cirrus which is about 0·17 mm. long and is slightly muscular. In entire mounts it appears almost globular on account of its thick musculature. Baer (1933) remarks that Osborne (1912), in his description of the species *C. marginatum*, claims that the cirrus is the pars prostatica, since it is surrounded by numerous cells. The writer agrees with Baer (1933) in that the cells surrounding the cirrus are simply myoblasts and that a real pars prostatica is lacking in Clinostomatidæ.

The ovary is somewhat irregular body, measuring 0·185–0·28 × 0·23–0·25 mm., and lies on the right side, internal to the right intestinal cæcum, almost midway between the two testes. The oviduct is a long thin duct which winds about considerably in the inter-testicular area. At a short distance from its origin, it gives out a fairly long Laurer's canal. The shell gland is small and situated immediately behind the ovary. The vitellaria consist of very small follicles extending in the inter-cæcal area behind the ventral sucker. A thick utero-duct is seen in the inter-testicular area and curves round the anterior testis on its right side. The utero-duct meets the uterus centrally, slightly in front of the anterior testis. The uterus is 2·88–3·3 mm. long and passes anteriorly in the middle line to a distance of 0·61–0·65 mm. behind the ventral sucker. The terminal portion of the uterus, the metraterm, is a stout duct, lined internally with a thick cuticle, and opens into the genital atrium situated on the right side of the anterior testis.

It is apparent from the foregoing description that the form in question is the metacercarial stage of *Clinostomum* sp. According to Price (1938) there are only five recognized species of the metacercaria of *Clinostomum, viz., C. pseudoheterostomum, C. dictyotum, C. chrysichthys, C. delagi* and *C. piscidium.* Referring to the key, it is seen that the form from *Notopterus notopterus* approximates to *C. delagi* Tubangui (1933). It can, however, be distinguished from it by the larger size and shape of the body, the relative size of the two suckers, the deeper indentations of the intestinal cæca, the branched nature of the testes, the position of the genital glands, the size and position of the cirrus sac, the situation of the shell gland and the length of the uterus. These points of difference being sufficient, it is proposed to regard the species described here as a new one, for which the name *Clinostomum indicum* is suggested.

Specific diagnosis of *Clinostomum indicum* Bhalerao, 1940

Metacercarial form. Length 9·5–11 mm. Maximum breadth 3·5–4·7 mm. Cuticle—spiny. Oral sucker 0·475–0·67 × 0·375–0·57 mm. Pharynx absent. Intestine with well-developed diverticula. Ventral sucker 0·86–0·118 × 0·82–0·96 mm. Genital pore on the right side of the middle of anterior testis. Genital glands in the middle of posterior half of body. Testes large and branched. Cirrus sac 0·8–0·84 × 0·28–0·35 mm. Ovary 0·185–0·28 × 0·23–0·25 mm. Vitellaria extending behind ventral sucker. Uterus 2·88–3·3 mm. long.

Host.—Notopterus notopterus.

Location.—Subcutaneous tissue.

Locality.—Poona.

REFERENCES

Baer, J. G. .. "Note sur un nouveau Trematode, *Clinostomum lophophallum* sp. nov. avec quelques considerations generales sur la famille des Clinostomidæ," *Rev. suisse Zool.*, 1933, **40**, 317–42.

Osborne, H. L. .. "On the structure of *Clinostomum marginatum*, a Trematode parasite of the frog, Bass and Heron," *J. Morph.*, 1912, **23**, 189–229.

Price, E. W. .. "A redescription of *Clinostomum intermedialis* Lamont (Trematoda : Clinostomidæ), with a key to the species of the genus," *Proc. Helminth. Soc. Wash.*, 1938, **5**, 11–13.

Yamaguti, S. .. "Studies on the Helminth Fauna of Japan, Part 2. Trematodes of Fishes I," *Jap. J. Zool.*, 1934, **5**, 249–541.

THE PANJAL TRAPS: ACID AND BASIC VOLCANIC ROCKS

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(Communicated by Prof. L. Rama Rao, F.A.Sc.)

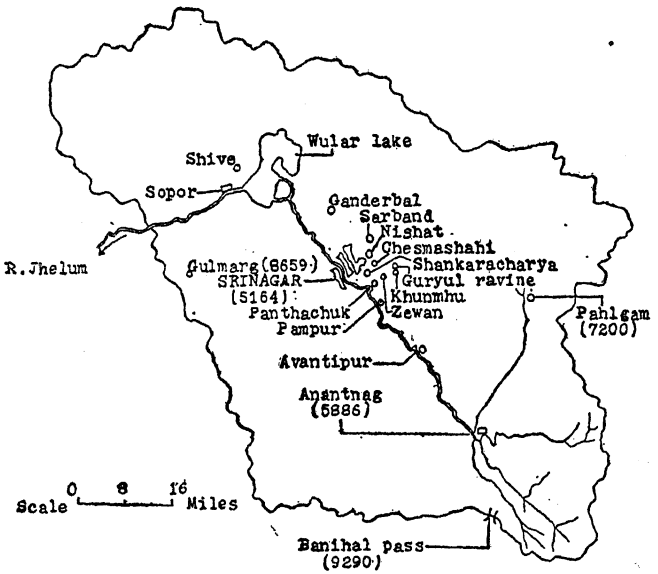
THE material on which this paper is based was collected by the author, mainly during the field work done in the summer of 1937 under the very valuable guidance of Prof. Raj Nath and partly alone, again in the summer of 1941. The material was collected from various localities in the vicinity of Srinagar and at such far off places as Gulmarg, Banihal pass, Pahlgam and Shive (near Sopore). The work was carried out in the Geology Department of the Benares Hindu University where the material is preserved.

Towards the close of the last century Lydekker and McMahon (1883, p. 218) established the volcanic origin of the Panjal traps. Middlemiss (1910, p. 235) described these rocks as "Genuine old basic lava flows". The late Professor K. K. Mathur (1933, p. 126) in a letter to *Current Science* described a specimen of rhyolite occurring in the Panjal traps at a locality named Panthachuk near Srinagar. About this discovery West (1935, p. 492) remarked "Although these rhyolites may be quantitatively unimportant, nevertheless their discovery is of much interest." Mr. D. N. Wadia (1939, pp. 412–13) describes these traps as "A basic variety of augite andesite or basalt of acidity varying 49% to 60% of a prevailing dark or greenish colour.....Acid and intermediate differentiation products also occur locally and in small masses, *e.g.*, trachyte, ceratophyre, rhyolite, acid tuffs, etc."

The Panjal traps generally overlie the Agglomeratic slate series of Upper Carboniferous age but at most places these lie unconformably over much older rocks. The upper limit is also different at different places. Sometimes these are found overlain by Lower Permian beds, as in the Vihi district where the traps are overlain by Gondwana plant bearing beds, while at other places the volcanic activity continued till Upper Triassic.

The presence of the acid rocks in the Panjal traps made their study very interesting. The author, therefore, studied this problem in greater detail. The results obtained clearly prove the true nature of acid volcanic rocks and their occurrence on a much larger scale than that previously thought of.

The trap rocks were studied at Cheshmashahi, Sarband to Nishat along the canal road behind the Moghul Gardens, Panthachuk, Khunmhu, Zewan, Avantipur, Pahlgam, Banihal pass, Gulmarg, Ganderbal and Shive. The relative position of these places is shown in the outline map of Kashmir (Text-Fig. 1).



TEXT-FIG. 1. Outline sketch map of Kashmir showing the localities where the Panjal trap rocks were studied

The acid volcanic rocks have been found occurring in great amount at the localities of Panthachuk, Avantipur and Cheshmashahi. At other places the basic volcanic rocks predominate.

At Panthachuk, the Panjal trap formation appears to show a sort of bedded structure and each bed may have been an individual outflow of lava. These beds are of different thickness and the bedding planes merge one into another after a short distance which makes their individual study rather difficult. With the idea of finding variation in constituents, specimens were collected at intervals of different heights from the base of the formation to its top and from what appeared to be different beds of lava flows. Near the top there is a vein formed of calcite and quartz and about 20 ft. below this there is an another vein having similar constituents. The specimen K/P6 lies immediately below the lower vein. The specimens K/P3–K/P6 were taken at intervals of different heights (Text-Fig. 2) one over the other.

The Acid Volcanic Rocks

In hand specimen the acid volcanic rocks are compact and greyish n colour. Quartz can be easily seen with the help of a lens. The specific ravity varies from 2·69 to 2·79 at the two extremes.

Under the microscope these rocks (Figs. 1, 2 & 3) are porphyritic. Among the phenocrysts euhedral to subhedral crystals of quartz are quite bundant. Crystals of alkali felspars are present in fairly large amount. Some of these are turbid. A few crystals of plagioclase felspars showing olysynthetic twinning are also present in some specimens (P/4 and K/C1 rom Panthachuk and Cheshmashahi respectively). A general character- tic, however, is the presence of green chloritic matter produced as a result f alteration. The chlorite usually occurs in association with felspars. he ground mass is usually turbid and is crypto- to micro-crystalline and hows crystals of quartz and felspar at places (Fig. 3). In a specimen from heshmashahi (K/C1) the ground mass is glassy and shows a sort of flow tructure. Magnetite and ilmenite are present in fairly large amount. In specimen from Panthachuk (K/P5) veins filled with secondary quartz are resent.

Seven rocks were analysed, five from Panthachuk and one each from heshmashahi and Avantipur. The values of analyses and norm are given n Tables I and II.

The Basic Volcanic Rocks

These rocks are compact and dark green in appearance. Small pieces f ferromagnesian minerals are present in sufficient quantity and can be een in hand specimen. The specific gravity of these varies from 2·8 to 3·0 t the two extremes.

Under the microscope the basic volcanic rocks (Fig. 4) are porphyritic. henocrysts of plagioclase felspars are present in fairly large amount. As a ule these rocks are very much altered and have undergone secondary silici- cation. Epidotisation is also a common phenomenon in these. Green hloritic product produced as a result of altertion is present in sufficient uantity. Big cavities filled with secondary quartz, secondary mica and pidote are fairly common. The ground mass is usually crypto-crystalline nd at places consists of minute needle-shaped prisms of felspar most of hich show polysynthetic twinning (*e.g.*, K/G4 from Guryul ravine). Iagnetite and ilmenite is present in fairly large amount. Specimens from ear Ganderbal are very much metamorphosed and show schistose tructure.

TABLE I

Analyses of Acid and Basic Volcanic Rocks

	Acid							Basic
	Panthachuk					Cheshmashahi	Avantipur	Sarband-Nishat Canal Road
	K/P3	K/P4	K/P5	K/P6	P/4	K/C1	K/A3	K/SN2
SiO_2 ..	66·87	64·96	66·22	64·89	68·43	63·59	64·99	57·37
Al_2O_3 ..	15·04	16·61	15·57	16·78	15·39	16·36	17·06	18·70
TiO_2 ..	0·90	0·94	1·00	1·08	0·93	1·09	0·93	1·16
MnO ..	0·04	0·09	0·05	0·06	0·03	0·02	0·06	0·10
Fe_2O_3 ..	1·47	1·97	2·77	1·54	1·41	1·58	1·94	3·24
FeO ..	2·46	2·99	2·61	2·62	2·84	2·86	1·76	3·88
CaO ..	3·36	2·87	2·72	3·28	1·99	4·45	3·44	5·86
MgO ..	1·03	1·10	1·21	0·98	0·66	1·44	1·32	2·64
Na_2O ..	4·22	3·85	2·36	4·68	3·68	5·32	3·52	3·75
K_2O ..	3·87	4·71	5·62	3·28	4·82	2·52	4·36	2·27
H_2O (-) ..	0·28	0·32	0·19	0·21	0·43	0·22	0·23	0·28
Total ..	99·54	100·41	100·32	99·40	100·61	99·45	99·61	99·25
Sp. gr. ..	2·77	2·74	2·70	2·73	2·69	2·76	2·75	2·77
Microscopic characters	Porphyritic. Fairly large phenocrysts of quartz. Ground mass glassy.	Porphyritic. Phenocrysts of quartz and alkali felspar. Ground mass more or less glassy.	Porphyritic. Phenocrysts of quartz and orthoclase felspar. Ground mass contains crystals of quartz and felspar. Secondary quartz present in cavities.	Porphyritic. Phenocrysts of quartz and orthoclase felspar. Ground mass more or less glassy.	Porphyritic. Phenocrysts of quartz, alkali and a few plagioclase felspar. Ground mass mostly glassy with a few crystals of quartz and felspar.	Porphyritic. Much decomposed. Phenocrysts of alkali and a few plagioclase felspar. Ground mass glassy showing flow structure. Cavities filled with secondary minerals are common.	Porphyritic. Phenocrysts of quartz only. Ground mass mostly glassy with a few quartz and felspar crystals.	Porphyritic. Phenocrysts of plagioclase felspar only. Ground mass contains needle-shaped crystals of felspar. Cavities filled with secondary minerals very common.
Analyst	P. N. Ganju							

TABLE II

Norm of Acid and Basic Volcanic Rocks

	Acid					Basic		
	Panthachuk					Cheshma-shahi	Avantipur	Sarband-Nishat Canal Road
	K/P3	K/P4	K/P5	K/P6	P/4	K/C1	K/A3	K/SN2
Quartz ..	19·32	15·84	22·92	16·20	22·32	12·24	17·46	9·66
Orthoclase ..	22·79	27·80	33·36	19·46	28·36	15·01	27·24	13·34
Albite ..	35·63	32·48	19·91	39·30	31·44	45·06	29·86	31·44
Anorthite ..	10·56	14·17	13·34	15·00	10·01	13·06	16·95	27·52
Corundum ..	..	..	0·70	..	0·41	..	..	..
Diopside ..	5·00	..	..	1·14	..	7·23	..	1·35
Hypersthene ..	1·99	5·07	4·05	3·65	3·61	2·19	3·43	8·70
Magnetite ..	2·08	2·78	3·94	2·32	2·09	2·32	2·78	4·64
Ilmenite ..	1·67	1·82	1·82	2·12	1·67	2·12	1·82	2·12
Water ..	0·28	0·32	0·19	0·21	0·43	0·22	0·23	0·28
Total ..	99·32	100·28	100·23	99·41	100·34	99·45	99·77	99·05

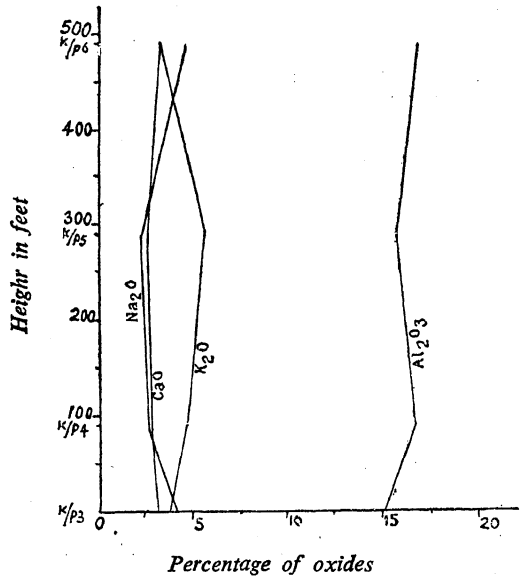
Only one rock sample K/SN2 from Sarband-Nishat Canal road was analysed. The values of analyses and norm are given side by side with the acid volcanic rocks in Tables I and II, for comparison.

Discussion

From the microscopic description, chemical analysis and other characters of Panjal traps occurring at Panthachuk, Avantipur and Cheshma-shahi, it is quite clear that acid volcanic rocks of the nature of rhyolites, trachytes and dacites do occur in the Panjal traps. That these acid rocks have not been formed by secondary silicification of andesites is also quite clear from the photomicrographs given (Figs. 1–3) where crystals of original quartz as well as those of the alkali felspars can be very clearly seen. A comparative glance at the analysis values of acid and basic rocks also proves beyond doubt the true nature of these acid rocks. It is true that most of the basic rocks have undergone secondary silicification, the secondary quartz being present in veins and amygdales but it is clearly different from the primary quartz seen in true acid volcanic rocks.

At Panthachuk, as already stated, specimens were collected at different heights from the base of the formation. Some of these were analysed chemically (see Table I) and the variation of various oxides with height has

been plotted (Text-Fig. 2). As is clear no definite change can be observed and the variations seem to be more or less irregular. The calcite and quartz veins at Panthachuk may have been formed by the steam and carbon dioxide evolved during the eruptions. These together may have decomposed the felspars producing calcium carbonate and silica deposited in the form of veins.



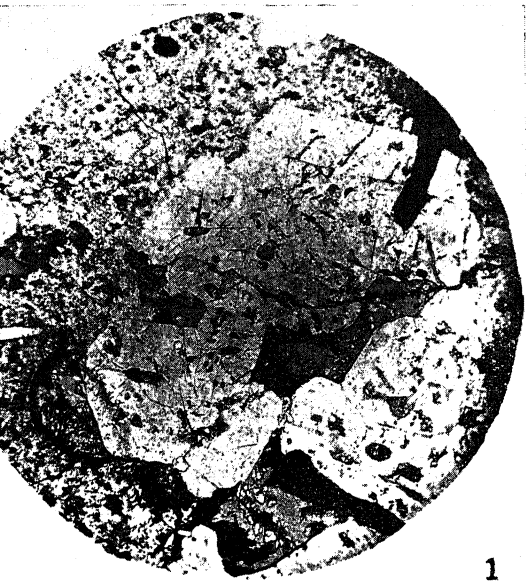
TEXT-FIG. 2. Graph showing the variation of different oxides with 'height' at Panthachuk

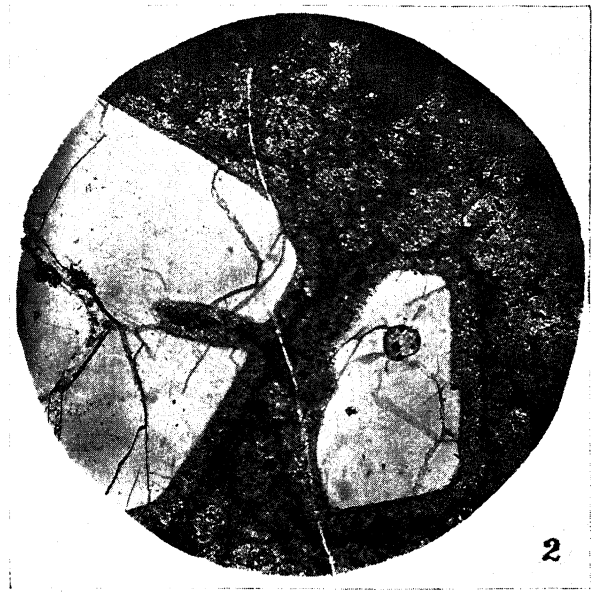
The acid rocks, however, show higher specific gravity than is usual. This may be explained as due to the high quantity of magnetite and ilmenite that these have been observed to contain.

As regards the quantitative importance of these, the author has traced the outcrop of acid rocks at three places within the distance of about 24 miles from Cheshmashahi to Avantipur. This shows that quantitatively these occur on a fairly large scale and it is likely that these occur on a much greater scale in Pir Panjal and other hills. It will be very interesting to get an idea of the total amount of the acid rocks as compared to basic rocks, their age, the time interval between the two outflows and their origin. The author hopes to continue the study of these points in detail.

Conclusion

The presence of rhyolites in the Panjal traps at Panthachuk was first brought to notice by late Prof. K. K. Mathur. The author studied the





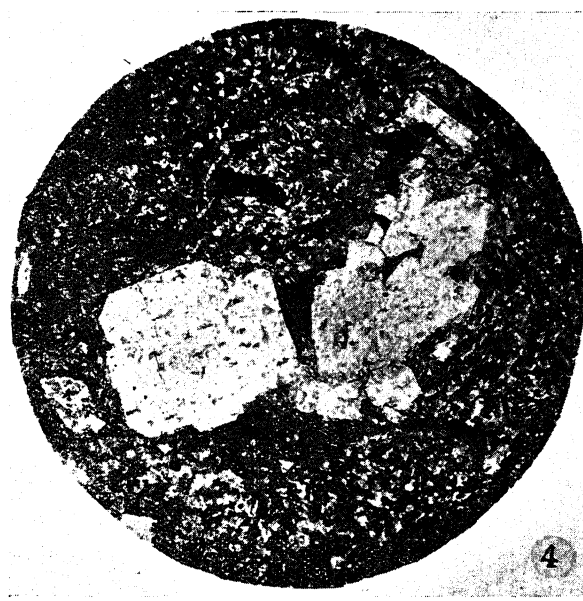


Fig. 1. Acid volcanic rock from the base of the quarry at Panthachuk, Srinagar, Kashmir. No. K/P 3.

Fig. 2. Acid volcanic rock from a height of about 300 feet at Panthachuk quarry. No. K/P 5.

Fig. 3. Acid volcanic rock from the Panjal trap formation at Avantipur, Kashmir. No. K/A 16.

Fig. 4. Basic volcanic rock from the Panjal trap formation along the Sarband-Nishat Canal road, Srinagar, Kashmir, No. K/SN 2.

Panjal traps in some detail and as a result the acid rocks have been found to occur at two other places also, *viz.*, at Cheshmashahi and Avantipur. These acid volcanic rocks are of the nature of rhyolites, trachytes and dacites and are very clearly different from basic volcanic rocks like andesites and basalts. These rocks are fairly abundant and must have played an important part in the Panjal volcanic activity.

Acknowledgment

I wish to express my great indebtedness to Prof. Raj Nath, M.Sc., Ph.D., D.I.C., for kindly suggesting this problem and the very valuable guidance he gave me throughout the work.

REFERENCES

Lydekker, R. .. "The geology of the Kashmir and Chamba territories and the districts of Khagan," *Mem. Geol. Sur. Ind.*, 1883, **22**, 211–24.

Mathur, K. K. .. "The Panjal Traps," *Curr. Sci.*, 1933, **2**, No. 4, 126.

——— .. "Panthachuk (Srinagar, Kashmir) Rhyolite," *ibid.*, 1935, **3**, No. 10, 492.

Middlemiss, C. S. .. "A revision of the Silurian-Trias Sequence in Kashmir," *Rec. Geol. Sur. Ind.*, 1910, **40**, 232–36.

Wadia, D. N. .. *Geology of India*, 1939, 410–14.

West, W. D. .. "Panthachuk (Srinagar Kashmir), Rhyolite," *Curr. Sci.*, 1935, **3**, No. 10, 492.

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STUDIES ON THE CORPUS LUTEUM IN *RHINOBATUS GRANULATUS* CUV.

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CONTENTS

Introduction

ALTHOUGH considerable work has been done on the histology and development of the corpus luteum, a great deal of controversy still exists regarding its formation, and the origin of the luteal cells. Hitherto, the subject has been mainly studied in relation to mammals and our knowledge of the organ in the lower vertebrates, especially in fishes is very poor. It was therefore felt that a study of the corpus luteum in the Elasmobranchs would be well worth an investigation.

Previous History

Various views have been held in regard to the mode of formation of the corpus luteum and the origin of the luteal cells in mammals. Von Baer (1827), the first author to discuss the formation of the corpus luteum, believed the organ to have an exclusive connective tissue origin, the follicular epithelium not having any share in its formation. The hypothesis of Paterson (1840), that the structure was formed from the blood coagulum left in the lumen

of the Graafian follicle, after escape of the egg, gained practically no support. Bischoff (1842) came to the conclusion that the luteal cells are entirely derived from the follicular epithelium, which undergoes remarkable hypertrophy and histological changes. He adduced further evidence in support of his theory by his subsequent study of the ovaries of the dog, guinea-pig and the roe. His work is of great importance for he is one of the few early workers who studied all the stages in the formation of the corpus luteum. The theories of Von Baer and Bischoff received considerable support from subsequent investigators.

Extensive work has been done on the corpus luteum of various mammals such as the mouse, rabbit, guinea-pig, *Tarsius*, *Tupaia*, *Sorex*, Sow, rat, marmot, sheep, ferret, bat, monkeys, marsupials and monotremes and certain reptiles by various authors. It is not proposed to review in detail the vast literature relating to the formatin of the corpus luteum in all cases, as complete references have been given by many authors like Van der Stricht (1912), and Marshall (1906 and 1922) although important papers must be referred to. Sobotta (1896) was the first author to make a systematic study of the formation of the corpus luteum by applying experimental methods which went far to confirm the view held by Bischoff. Among the supporters of Bischoff's theory we find Heape (1897), Stratz (1898), Kreis (1899), Belloy (1899), Honore (1900), Sandes (1903), Cohn (1903), Völker (1904), Marshall (1904), O'Donoghue (1911, 1914 and 1916), Robinson (1918), Hill and Gatenby (1926) and Deanesly (1930) all of whom agree that the follicular epithelial cells hypertrophy and give rise to luteal cells.

After the publication of Sobotta's important work, several investigators have strongly supported the theory originally put forward by Von Baer and as adherents to his theory may be mentioned Clark (1898), Doering (1899), His (1899), Paladino (1900), Nagel (1899), Jankowski (1904) and Williams (1904).

Apart from these three hypotheses, a fourth was put forward by Schrön (1863), and was supported by Rabl (1898), Leo-loeb (1906), Meyer (1911), Van der Stricht (1901 and 1912), and more recently by Corner (1915 and 1919). These were of opinion that the luteal cells of the mature corpus luteum are derived from both the follicular epithelium and the cells of the theca interna. In this connection mention may be made of some of the more important workers like Miller (1914), Novak (1921), Gatenby (1924) who have done work on human corpus luteum.

To Giacomini (1896) belongs the credit of being the first to study the gland in Elasmobranch fishes. He found a well-marked glandular organ

very similar to the corpus luteum of mammals in *Myliobatus bovina* which he described as a more or less solid body in which the enlarged epithelium is penetrated by an extensive ingrowth of connective tissue and blood vessels so that the corpus luteum appears as a " glandular organ full of epithelial tubes ". Buhler (1902) was unable to discover any great hypertrophy of the follicular epithelium in the spent follicles in cyclostomes and certain teleosteans. Cunningham's (1898) observation on the ruptured follicles of teleosteans led him to similar conclusions. Wallace (1904) drew our attention to the peculiar form assumed by this body in *Spinax niger*, which shows certain resemblances to the mammalian corpus luteum. Regarding the ruptured follicles of the ovary of *Zoarces*, Wallace states that the follicular epithelium undergoes a slight hypertrophy by simple enlargement of its cells but the follicle does not show any further advancement.

Material and Methods

Rhinobatus granulatus is a typical ovoviviparous Elasmobranch like most Batoids. The material for the study was taken from a female specimen collected in August 1940 from the Madras Coast. The ova were large and the two ovaries contained eggs in all stages of development. In each ovary, over twenty corpora lutea of varying stages from the freshly ruptured follicle to the fully developed solid, glandular corpus luteum were present. The ruptured openings of these follicles through which the egg had escaped to the exterior had closed up. The advanced corpora lutea were yellowish in colour while the freshly ruptured follicles were coloured pinkish white.

The corpora lutea were preserved by several methods. The fixatives successfully employed were Bouin, Carnoy, Regaud, Champy, Flemming, 5% formalin and corrosive sublimate. The material was dehydrated and cleared in cedar wood oil in the usual manner and paraffin method of embedding was employed in all cases. Sections were cut 4 to 7 microns thick. The stains employed were iron hæmatoxylin and Mallory's triple stain. Of these Heidenhain's iron hæmatoxylin was the stain most frequently used and was found to give the best results.

The Follicular Wall of the Ripe Ovum

The ripe ovum is enveloped by the egg membranes and a clearly delimited outer follicular wall. The former, as in all Elasmobranchs, consists of the inner zona radiata and the outer vitelline membrane which is closely attached to the zona radiata and lies immediately inside the follicular epithelium.

In the fairly ripe condition of the egg the follicular wall consists of three layers—the follicular epithelium, the membrana propria and the theca folliculi (Ph. M.1). The most remarkable feature in *Rhinobatus granulatus* is the presence of two kinds of cells in the follicular epithelium. This layer is quite distinct and delimited from the theca folliculi. It consists of a large number of small cylindrical cells intercalated among which are a small number of large vesicular cells. Of these two sets of cells, the smaller cells are long and columnar with oval nuclei densely filled with deeply staining fine chromatin granules and a well marked nucleolus. The bigger cells have clear cytoplasm and more or less central vesicular nuclei. These nuclei are lightly staining and possess a distinct nucleolus but a peripheral chromatin reticulum is indistinct. The resemblance of these large cells to small oocytes is striking. Semper (1875) went to the extent of suggesting that these peculiar large cells were primitive ova destined to become permanent ova. Giacomini (1896) in describing the follicle of *Chimæra* mentions that the epithelium consists of large cells scattered among which are small cylindrical cells. *Rhinobatus granulatus* resembles *Raia*, *Scyllium* (Balfour, 1878), *Torpedo* (Schultze, 1875, as stated by Wallace, 1904), *Myliobates*, *Trygon* (Giacomini, 1896) and *Chimæra* (Wallace, 1904) in this feature of possessing two kinds of cells in the follicular epithelium. But it differs from the condition in *Chimæra* in the larger cells being comparatively fewer. The membrana propria is a very thin layer of elongated cells closely investing the follicular epithelium.

Surrounding the follicular epithelium is a layer of connective tissue—the theca folliculi. This layer is readily distinguishable from the follicular epithelium and consists of a fairly compact internal portion formed of connective tissue cells and fibres and an external portion of more protoplasmic and elongated cells loosely scattered and almost parenchymatic in nature. These are the theca interna and theca externa. In the higher vertebrates the connective tissue theca also shows a differentiation into the theca interna and theca externa. This distinction becomes very much more marked after the rupture of the follicle. The theca interna is 5 to 10 cells in thickness and at its maximum is about twice as thick as the follicular epithelium. The cells are smaller than the small cells of the follicular epithelium and their cell boundaries generally indistinct. Their nuclei are oval or rarely spherical and possess a distinct reticulum and a small nucleolus. The cytoplasm is faintly alveolar in texture and stains lightly. Fine connective tissue fibres run amongst them. These layers of cells and fibres are disposed parallel to one another (Ph.M.l).

The theca externa is thinner and consists of loosely arranged elongated cells and fibres. Thin-walled blood vessels occur in this outer region. The endothelium, surrounding the theca is not well defined. Wedged in amongst the concentrically arranged elements of the theca externa are groups of small cells with deeply staining spherical nuclei and with indistinct cell boundaries. The cells have probably originated from the stroma cells and have remained unaltered.

The follicular wall of the fully mature egg shows that the columnar cells of the follicular epithelium have increased in number. The nucleus has assumed a more vesicular form and the cytoplasm has become more granular and deeply staining. There is a reduction in the number of the large cells. In the theca interna the connective tissue cells show a tendency to group themselves round small spaces giving the appearance of tubules in section (Ph. M. 2 and Text-Fig. 1). The cells constituting the tubules have slightly increased in size and the nucleoli have become more distinct. The connective tissue fibres run in between these tubules but they still retain their parallel arrangement.

The theca externa is a thin concentrically disposed layer surrounding the theca interna. The cells constituting this layer are highly protoplasmic. Cell boundaries cannot be made out. A slight increase in the number of blood vessels as well as in the free thecal cells in this layer has been observed.

Corpus Luteum

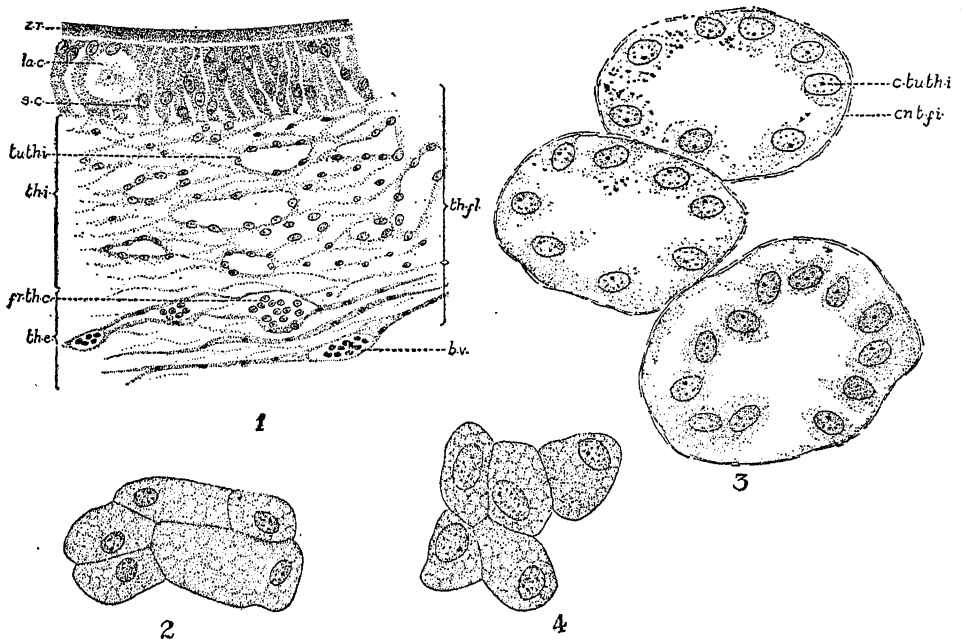
Stage I.—The corpora lutea are elongated flask-shaped bodies the mouth closed early in development with the ingrowth of the surrounding connective tissue. No stage showing the actual opening or rupture was available. The corpora lutea are very much smaller than the ripe unruptured follicle and it is evident that considerable contraction has taken place by the collapse of the wall as a result of the rupture and subsequent escape of the ova. The changes undergone by the follicular wall of the corpus luteum resulting in the final formation of the solid gland is described from within outwards in the following account.

Photomicrograph 3, shows a low power view of portion of a transverse section of the corpus luteum. There is a central lumen of considerable size containing a few globules of a darkly staining substance, probably yolk granules left behind after the escape of the egg. The detailed structure of the follicular wall is illustrated in Photomicrograph 4. The three layers of the follicular wall have undergone marked histological changes. The follicular epithelium is thrown into folds due to the escape of the ovum and the

consequent contraction of the follicular wall. It has also undergone considerable change and exhibits a multilayered appearance in contrast to its previous single-layered condition. Soon after the rupture, the follicular cells undergo enormous hypertrophy. Mitotic divisions have been described in the follicular epithelial cells in the early stages of the formation of the corpus luteum in the case of certain marsupials (O'Donoghue, 1914, 1916), in sheep (Marshall, 1904) and in certain lizards (Weekes, 1934). In the present form, however, such divisions have not been observed in the epithelial cells and the multilayered condition must have been brought about mainly by the hypertrophy of the follicle cells together with the contraction the follicle undergoes due to the escape of the egg. Instead of there being two sets of cells, small and large, the follicular epithelium is now composed of a more or less continuous layer formed by the hypertrophy of the small cells now arranged in varying thickness of 1–4 cells. The big cells have now completely disappeared. The disappearance of the large cells has been noticed in reptiles and is regarded by Hett (1924) as being absorbed by the egg, being of the nature of nutritive cells. Wallace (1904) is of opinion that the large specialised cells of the follicular epithelium of *Chimæra* are "nutritive" cells and they degenerate and disappear before the maturation of the egg.

The follicular epithelium is separated from the connective tissue theca probably due to the pressure caused by the contraction of the wall after ovulation. The cells forming it are deeply staining varying in shape from a polyhedral to a spherical condition with distinct cell-boundaries (Ph. M. 5 and Text-Fig. 2). They have rather large spherical or oval deeply staining nuclei which typically contain one or two nucleoli and a well marked reticulum of chromatin granules. The cytoplasm is moderately stained.

The connective tissue sheath is very different from that of the unruptured follicle and the distinction between the theca interna and theca externa is well marked (Ph.M. 4). The theca interna follows the wavy contour of the follicular epithelium and is seen to send long finger-shaped projections of connective tissue fibres and cells in between the folds of the epithelium. There is great increase in size and numbers of the tube like structures of the theca interna (Text-Fig. 3 and Ph.M. 6). The cells constituting the tubules have greatly increased in size but at this stage they are smaller than the hypertrophied follicular epithelial cells. They have definite oval nuclei with distinct nucleoli and a well-marked reticulum with peripheral chromatin granules. In a few cases the boundaries of these cells can be made out. On a careful examination the connective tissue fibres are now seen to have wedged themselves all round the tubules closely



TEXT-FIGS. 1–4. *Rhinobatus granulatus* Cuv.—Fig. 1. T. S. of a fully mature egg showing the structure of the follicular wall. ×400. Fig. 2. A portion of the follicular epithelium (Stage I) magnified to show the hypertrophied follicular epithelial cells. ×900. Fig. 3. A few tubules of the theca interna (Stage I) showing the theca interna cells and the fibres investing the tubule. ×900. Fig. 4. A portion of the follicular epithelium (Stage II) showing the follicular epithelial luteal cells. ×900.

investing them and giving the appearance of definite walls to the tubules. The formation of these tubule-like structures in the theca interna is of considerable interest from the point of view of comparative histology as I am not aware of any record of such structures in the theca interna in other groups of animals.

The theca externa has but slightly increased in thickness. There is however an increase in the number of blood vessels. A few blood capillaries invade the theca interna and run in between the above-mentioned tubules. The groups of small spherical free thecal cells with darkly staining nuclei and clear cytoplasm have definitely increased in number, and some of them have penetrated in between the tubules of the theca interna.

Stage II.—A transverse section of the corpus luteum at this stage of development shows considerable resemblance to the previous stage and so the more important differences alone need be enumerated. The central lumen is further reduced. The folded appearance of the follicular

epithelium is still retained. No marked increase in number of the epithelial luteal cells is noticed and their histological character has altered but slightly. The majority of the cells are oval or spherical in shape and are now slightly smaller than in the preceding stage (Text-Fig. 4). The cytoplasm stains lightly and appears reticulate. The nuclei, mostly eccentric in position, contain a large irregular mass of chromatin or karyosome and a few smaller nucleoli with all of which a well-marked reticulum studded with small chromatin granules is associated.

The connective tissue theca takes an important part in the formation of the early corpus luteum. It has further thickened and the tubular structures in it have now become very definite and slightly more numerous. No great increase in the size of the cells of the thecal tubules is noticed but in the majority of them the cell-boundaries have become clearly marked (Text-Fig. 5). They continue to be definitely smaller than the epithelial luteal cells. The nuclei and nucleoli are perfectly distinct and moderately stained.

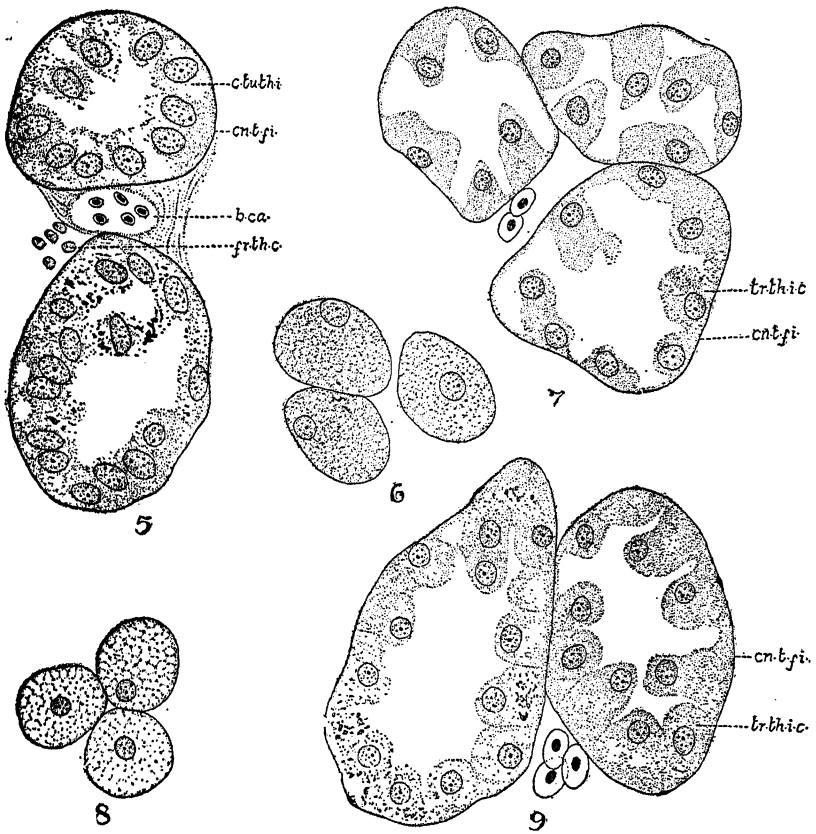
An advance is also shown in the commencement of ingrowths of strands of cells from the theca externa. There is a noticeable increase in the number of blood vessels in the theca externa and the projecting ingrowths above mentioned carry the blood vessels and groups of free thecal cells among the tubules of the theca interna.

Stage III.—A comparison with the previous stages shows a marked advance (Ph.M. 7). The central lumen is a little more reduced in size. Appreciable changes have taken place in the follicular epithelium. The folded condition has disappeared on account of a breaking up in the continuity of the layer and there is only a faint suggestion of the original folds. The epithelial luteal cells show a tendency to aggregate themselves into small masses or into more or less diffuse groups and most of them come to lie near the theca interna. They have assumed a more or less spherical shape in contrast to their previous columnar and elongated condition (Text-Fig. 6 and Ph.M. 8).

Accompanying these changes fine deeply staining chromatic granules have made their appearance in cytoplasm. Cytoplasmic vacuoles now begin to appear which owing to the large number of deeply staining granules are at first not readily distinguishable.

The theca interna is distinctly delimited from the follicular layer and still retains its folded appearance, though the interspaces between the folds become filled up by the approximation of the epithelial luteal cells (Ph. M. 7).

The more internally placed tubules have become larger in size than the superficial ones. The cells of the larger tubules have undergone further histological changes (Text-Fig. 7 and Ph.M. 9). The shape of these cells constituting the tubules is variable, but a number of them are spherical. The majority of the nuclei have become distinctly spherical and contain in addition to the nucleolus, a network of numerous chromatin granules, and



TEXT-FIGS. 5–9. *Rhinobatus granulatus* Cuv.—Fig. 5. A portion of the theca interna (Stage II) magnified to show the tubules of the theca interna, the blood capillaries and the free thecal cells. ×900. Fig. 6. Follicular epithelial luteal cells (Stage III). ×900. Fig. 7. The tubules of the theca interna (Stage III) showing the transforming theca interna cells. ×900. Fig. 8. A few follicular epithelial luteal cells (Stage IV). ×900. Fig. 9. The tubules of theca interna (Stage IV) magnified to show the transforming theca interna cells. ×900.

are of the same size but less deeply staining than those of the luteal cells of the follicular epithelium. The cytoplasm is rather dense and contains fine granules of very minute size. The fibres of connective tissue investing the tubules are quite distinct. There is a slight increase in the blood vessels

passing in between the tubules. An advance on the earlier stages is observed in the invasion of strands of connective tissue fibres along with blood capillaries and free thecal cells in between the groups of epithelial luteal cells. No appreciable change is observed in the theca externa.

Stage IV.—Photomicrograph 10 represents a portion of a transverse section of the corpus luteum at this stage. It is now slightly more advanced and is interesting in that the epithelial luteal cells show further cytological changes. The structure of the follicular wall is illustrated in Photomicrograph 11. The epithelial luteal cells form the conspicuous elements. They are distinguished both by the cytoplasmic vacuoles and by their large size and spherical shape (Text-Fig. 8 and Ph.M. 12). Though there is no noticeable increase in size of the epithelial luteal cells, an increase in number as compared with the previous stages is observed. The lumen is further reduced as a result of the luteal cells becoming more evenly spread out. The cytoplasm of the luteal cells is occupied by the spherical vacuoles which are much more marked than in the previous stage and consequently these cells are rendered more conspicuous than in the earlier stages examined. As a result of the great development of the vacuoles the chromatic granules are wedged in the intervacuolar cytoplasm. The nucleus does not show any change in its cytological details.

Another point of interest is that the theca interna cells constituting the inner tubules exhibit the first evident signs of transformation into luteal cells similar to those shown by the follicular epithelium (Text-Fig. 9 and Ph. M. 13). The first stage in the transformation of these cells into luteal cells is the appearance of chromatic granules together with an enlargement of the cytoplasm and a greater definition of the cell membrane. The majority of nuclei become spherical with distinct chromatin reticulum and deeply staining nucleolus. Cytoplasmic vacuoles begin to appear which owing to the large number of fine granules, are not readily distinguishable. The connective tissue fibres surrounding the tubules are well marked. The distinction between the cells of the theca interna and the epithelical luteal cells is maintained. There is an increase in the invasion of the blood capillaries and free thecal cells in between the tubules. The ingrowth of connective tissue fibres from the theca interna in between the groups of epithelial luteal cells initiated in the last stage has become more prominent.

The theca externa is thicker than in the earlier stage. This increase in the thickness of the connective tissue is accompanied by a corresponding increase of blood vessels. The invading strands of cells of the theca externa carrying blood vessels and free thecal cells into the theca interna are very prominent (Ph.M. 10).

Stage V.—This stage is interesting in that the follicular part and the theca interna has become indistinguishable (Text-Fig. 10 and Ph.M. 15). The luteal cells now almost fill the internal lumen, except for a narrow slit in the centre of the gland (Ph.M. 14).

The epithelial luteal cells have undergone further histological changes (Text-Fig. 11 *a* and Ph.M. 16). They are now polygonal or spherical with distinct large vesicular nuclei containing one or two nucleoli and a peripheral reticulum rich in chromatin granules. The vacuoles with which the cytoplasm is honey-combed have become smaller and spherical. Another change connected with the mature corpus luteum is the development of larger chromatic granules which take up an inter-vacuolar position. They are very much marked at the angles of the honey-combed vacuoles. Hence the inter-vacuolar regions take a deep stain which therefore emphasises the boundaries of the vacuoles so as to produce the conspicuous honey-combed appearance characteristic of the cytoplasm of the fully developed luteal cells. They have reached the height of their histological development and the main feature of this stage is that all the cells are in the same stage of activity. The vacuoles are very prominent and occur in every luteal cell and are so numerous that there is only little cytoplasm in the cell. In abundance and sharpness of outline and uniformity of structure, these vacuoles and their exquisitely honey-combed appearance are very striking. The ring-like vacuoles have been described in the luteal cells of higher mammals like Ungulates, Carnivora, Rodents and Primates. To my knowledge, they are now described for the first time in the luteal cells of an Elasmobranch. Simultaneous with the merging of the sheath portion and the follicular part there is an invasion of the connective tissue fibres a few of which surround the groups of inner epithelial luteal cells.

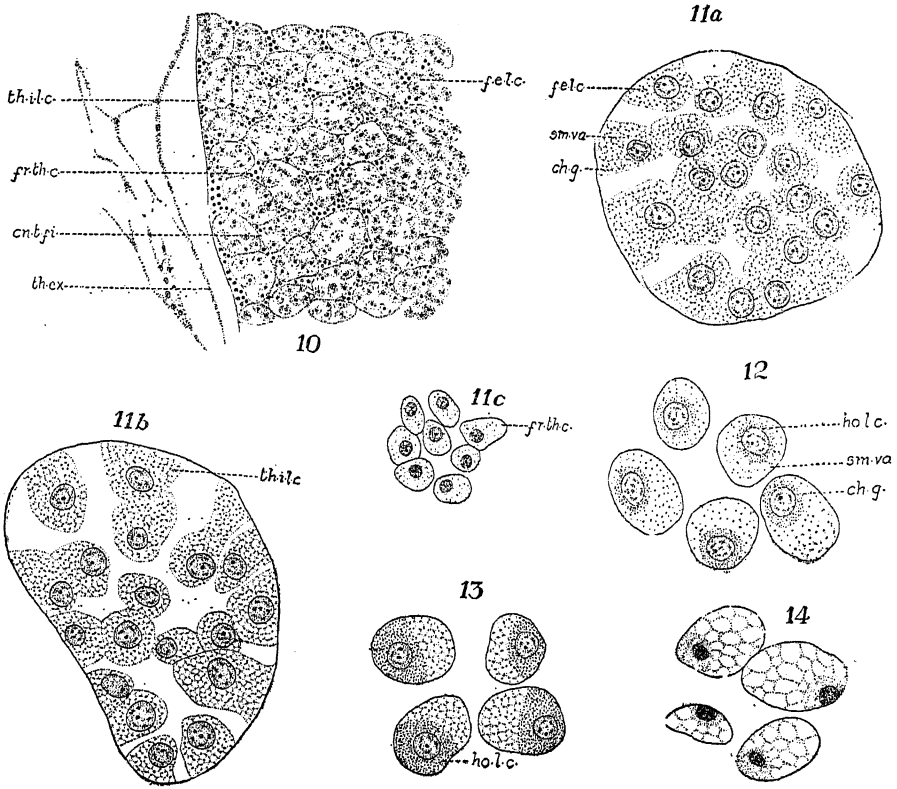
Towards the superficial region of the original theca interna the cells constituting the tubules are slightly smaller than the cells towards the inner region. The fibres investing these cells still persist round them retaining the tubular appearance. The smaller cells of these tubules pass imperceptibly into larger and more glandular luteal cells of the inner tubules. As a result of the enlargement of the cells composing the tubules some of them have been pushed into the lumen of the tubules giving them a solid appearance. A careful study of the cells of tubules of the theca interna reveals that they have undergone remarkable cytological changes. Text-Fig. 11 *b* and Ph.M. 17 illustrate the changes undergone by the cells during the metamorphosis of these thecal into luteal cells. Comparison with the earlier stages shows an increase in size of the cells of the theca

interna. With the transformation of the theca interna cells into luteal cells changes similar to those occurring in the follicular epithelial cells take place. The nucleus which hitherto has remained oval and small gradually enlarges, the charomatin granules getting concentrated in a uniform manner on the inner surface of the nuclear wall. When the nucleus has reached its maximum growth it presents a characteristic vesicular appearance (Text-Fig. 11 *b* and Ph.M. 17). With the assumption of luteal condition the nucleolus has by its special prominence become a distinct feature of the nucleus. The chromatin material as well as the nucleolus are distinct and deeply stained. The spherical vacuoles become very clear and the whole cytoplasm is honey-combed with ring-like vacuoles as in the epithelial luteal cells. The honey-combed appearance is due to mutual compression. Consequently there is no difference now in size or cytological structure between the luteal cells developed from the theca interna and the luteal cells formed from the original follicular epithelium.

The groups of free thecal cells may be easily noted in the corpus luteum. These are much more abundant and more conspicuous than in any of the earlier stages. The free cells of the theca can be clearly distinguished from the luteal cells by their smaller size, round, vesicular, deeply staining nuclei containing large amount of chromatin and by the uniformly staining cytoplasm (Text-Fig. 11*c* and Ph.M. 16). They are interspersed in between the groups of luteal cells. A careful study reveals the changes they have undergone. The cytoplasm is densely packed with minute vacuoles. These cells vary from round to oval shape and the nucleus generally has an eccentric position. The origin of these cells can be easily traced from the outer region of the theca folliculi. In the earlier stages they occur as clumps of theca cells situated in the periphery of the corpus luteum wedged in between the concentric layers of the theca externa. Later on they extend in between the tubules of the theca interna. Later still a small number grow along with the connective tissue fibres in between the diffuse groups of epithelial luteal cells. In the fully developed corpus luteum, these cells are found about the periphery and also along the connective tissue fibre ingrowths which penetrate and surround the groups of luteal cells. They also fit into the interstices between the groups of luteal cells. They form a special group different in histological character, location, and origin, from the luteal cells. The presence of a regular system of vacuoles very much like that found in the luteal cells suggests that these cells have a function similar to that of luteal cells.

The theca externa is very much reduced and consists of only a thin layer, about three cells deep, investing the corpus luteum. The luteal tissue

is held together by a framework of reticular connective tissue fibres which forms a dense network round the groups of luteal cells. There is an increase in the vasicular tissue in the theca externa and an invasion of blood vessels and free thecal cells into the luteal tissue and their extension along the connective tissue framework is clearly seen.



TEXT-FGIS. 10–14. *Rhinobatus granulatus* Cuv.—Fig. 10. T. S. of portion of corpus luteum (Stage V) showing the luteal tissue formed from the theca interna and the follicular epithelium and the penetration of connective tissue fibres and free thecal cells. ×80. Fig. 11*a*. A group of follicular epithelial luteal cells (Stage V) showing the elaborate system of cytoplasmic vacuoles and chromatic granules in the inter-vacuolar cytoplasm. ×900. Fig. 11*b*. A group of theca interna luteal cells showing similar cytoplasmic structures as the follicular epithelial luteal cells. ×900. Fig. 11*c*. A group of free thecal cells. ×900. Fig. 12. Luteal cells (Stage VI) magnified to show the changes in the cytoplasm. ×900. Fig. 13. Luteal cells (Stage VI) showing the homogeneous staining area of the cytoplasm. ×900. Fig. 14. Luteal cells (Stage VII) showing commencing degeneration by the appearance of large irregular vacuoles. ×900.

From a study of this stage it is clear that the follicular epithelial cells and the cells of the theca interna forming tubules enter into the formation of the luteal elements.

Stage VI.—In a still more advanced stage the corpus luteum has become solid throughout. It is a glandular organ composed of closely packed cells arranged in anastomosing columns and masses, between which run the blood vessels and groups of free thecal cells (Ph.M. 18). It is invested by a thin connective tissue envelope composed of protoplasmic cells and fibres of the theca externa. There is a general rupture of the tubules with the result that the luteal cells are somewhat spread apart, but are still held together by means of protoplasmic connections. The previous stage passes imperceptibly into this stage though a microscopic study shows that the cells constituting the corpus luteum exhibit a diversity in their cytoplasmic structure. Most of the luteal cells have an immense number of vacuoles of very constant size and shape as in the preceding stage. The intervacuolar spaces are broader and the chromatic granules are larger and stain deeply. A number of luteal cells show changes both in their shape and cytoplasmic structure as represented in Text-Fig. 12. The shape of the cells is variable. The cytological structure of these fully formed luteal cells is as follows. The nucleus is usually spherical and large but not so chromatic as in the previous stage though one or more nucleolar bodies and numerous small chromatin granules are present. It is noted that the cytoplasm as previously, is honey-combed with spherical vacuoles which are not so numerous as in the preceding stage. There is a homogeneous staining area round the nucleus while the vacuoles are confined to the periphery (Text-Fig. 12). It appears to consist of a group of very fine chromatic granules concentrated round the nucleus. These granules show up fairly with iron-alum and hæmatoxylin. In Mallory's triple stain they appear as light orange granules, diffusely scattered round the nucleus. It is also observed that the nucleus too is stained orange. In some of the cells the homogeneously staining granular area extends to the periphery (Text-Fig. 13). Regarding the nature of the chromatic granules of the luteal cells they may be of an albuminous nature since they are best preserved by mercury salts and are not dissolved by alcohol or water from fixed tissue. The presence of these granules recalls the condition of luteal cells as described by Corner (1915) in Swine.

The free thecal cells described in the previous stage show considerable increase in number but the cytological character of the cells remains unchanged. They become uniformly scattered about the luteal cells. In addition strands of protoplasmic cells could be seen growing from the theca externa more or less in the form of fine root-like processes in between the folds of the wall of the corpus luteum. In some places they can even be seen to penetrate into the central region. There is a great reduction in the theca externa.

Stage VII.—The histological character of the luteal cells of this stage has altered and careful examination shows that there is a marked increase in the homogeneous staining area of the cytoplasm with a corresponding reduction of vacuoles. Luteal cells in various stages, from a condition of full preservation having vesicular nuclei and honey-combed cytoplasm to stages of degeneration, could be noticed. Degenerating cells are now few and are found near the periphery of the corpus luteum. The vacuoles in the cytoplasm of the degenerating cells show variation in size and in some of the cells they become enlarged (Text-Fig. 14) accompanied by a reduction in size of the nuclei. In a number of cells the nuclei stain intensely. The boundaries of these cells can no longer be made out. Free deeply staining nuclei without the enveloping cytoplasm are occasionally found. A clumping of chromatin of the nucleus in the degenerating cells is observed as a result of which neither the nucleolus nor the chromatin granules are visible. In this connection it is interesting to note that the degenerating changes set in only late. The theca externa shows marked reduction and is seen as a very thin envelope of connective tissue cells and fibres surrouding the corpus luteum.

Discussion

We shall take the structures which finally form the corpus luteum in the order of their importance.

Luteal Cells.—The origin of the luteal cells in the different groups of vertebrates has been the subject of considerable amount of controversy. The general consensus of opinion at present seems to be that luteal cells are derived either exclusively from the follicular epithelium or from both the follicular epithelium and the theca interna. From the study of the formation of the corpus luteum in *R. granulatus* it is clear that the follicular epithelial cells and the theca interna cells which form tube-like structures develop and transform themselves into luteal cells of the fully developed corpus luteum. The theca interna cells which remain distinct from the follicular epithelial cells in the early stages, undergo interesting histological changes and metamorphose into luteal cells very much like those formed from the follicular epithelium so that in the fully developed corps luteum there is no difference between the luteal cells constituting the organ.

It has been shown that the small cells of the follicular epithelium of the unruptured follicle, after ovulation develop and form large spherical or polygonal glandular cells which exhibit similar cytoplasmic changes as in certain mammals. The large cells undergo reduction and finally, in the ruptured

follicle, disappear completely. The same phenomenon of decrease in numbers and final disappearance of the large cells has been noticed in the reptiles.

Corner (1915) recognises an endoplasm and exoplasm in the cytoplasm of the luteal cells in the Sow. With regard to the cytoplasmic changes undergone by the luteal cells this author distinguishes seven periods in the history of the corpus luteum of which the most important are the Preparatory, Exoplasmic, Transitional, Endoplasmic and beginning of retrogression. According to him the corpus luteum being an organic body each stage passes imperceptibly into the next stage. In the exoplasmic stage he describes the highest development of the exoplasmic region when the exoplasm is " occupied by a most curious and elaborate system of vacuoles". In the later stages a homogeneous staining area, the endoplasm, develops round the nucleus which gradually increases. As the endoplasmic zone increases there is a corresponding decrease of the exoplasmic area. Finally the whole cell is practically occupied by the homogeneous cytoplasm. He also observes that in the later periods the lipoid globules which form the regular vacuoles " have practically disappeared from the cell, being found if present, as a few globules at the periphery ". Still later " many cells contain one or two globules twice as large as the nucleus, in the cytoplasm ". The luteal cells of stages V, VI and VII of the present study correspond with the exoplasmic, endoplasmic and retrograssive periods respectively, of the Sow's corpus luteum. The fully formed luteal cells in *R. granulatus* are large, each cell having a large characteristic vesicular nucleus containing one or two large nucleoli and peripheral reticulum rich in chromatin granules. The cytoplasm becomes honey-combed with a regular system of spherical ring-like vacuoles in non-osmic fixatives.

It is interesting that the structure of the luteal cells and the cytological changes undergone by them in *Rhinobatus granulatus* agree in important respects with those of Sow, described by Corner (1915) in spite of their widely separated position in the vertebrate series.

Cohn (1903) was the first author to describe these remarkable vacuoles of the luteal cells in the corpus luteum of the rabbit. These ring-like bodies in the luteal cells have been described in such higher mammals as Ungulates, Carnivores, Rodents and Primates. Although Van der Stricht (1912) describes in detail the secretory appearance in the corpus luteum of bats he does not mention the ring-like structure in the luteal cells. Clark (1898) who studied the luteal cells of swine says that they become full of vacuoles and their cytoplasm shrunken. Regarding this statement of

Clark, Corner (1915) says "he was probably actually describing the exoplasmic vacuoles, which we shall see to be evidences of cellular activity, not of senescence".

Practically all workers agree on the point that "the deposition of fat in the luteal cells is not a sign of degeneration but represents a normal function of an endocrine nature". Most of them distinguish two periods in the deposition of fat in the luteal cells. A detailed study of the fatty inclusions in the luteal cells of bats is made by Van der Stricht (1912). The author recognises two periods in the deposition of fat, the first being 'epithelial in nature representing a secretion of the corpus luteum, but that the later reappearance of presumably fatty material is a sign of senescence in the tissue'. Corner's (1915) observations on the luteal cells regarding the nature of deposition of fat is in close agreement with that of the above author. Corner (1915) says 'the later deposition of fat I take to be a sign of senility of the tissue whereas the large amount of lipoid in the early lutein cell would seem to be correlated with physiological activity'.

Gatenby (1924) working on the human corpus luteum of ovulation is of opinion that the lutein granules are lipin and not true fat since "they do not occur in the same irregular manner in which true fat appears in cell; they do not vary much in individual size; they do not always reduce the osmic acid vigorously and finally they correspond to position with the expected arrangement of the mitochondria". This statement of Gatenby's is in agreement with the views of Cesa-Bianchi (Gatenby, 1924). According to Gatenby (1924) the mitochondria swell in size and become loaded with lipochrome which gives the corpus luteum its characteristic appearance. He concludes that since the lutein granules are disposed in the same manner as the mitochondria and all the granules are of equal size they are formed from the mitochondria. As further evidence in support of this view he states that in no case "the neutral fat appears within any cell in the form of chains of minute granules all of the same size".

Owing to the lack of specimens it has not been possible to study the cytoplasmic structures, *viz.*, mitochondria and golgi and the secretory products of the luteal cells. However, the fact that all the vacuoles of the cytoplasm are regular in arrangement and equal in size, point to the conclusion that the globules formed in the early active stages are lipoid in nature. It may be suggested that the lipoid globules formed become surrounded by chromatic granules which are also present in the cytoplasm and the former are readily removed by the reagents employed in fixing, and dehydration. Hence the position of the lipoid globules are indicated by rows of ring-like

vacuoles with chromatic granules in the inter-vacuolar cytoplasm. The appearance of large vacuoles at the periphery of the cell in the last stage without the uniformity of size observed in the earlier stages may be due to the formation of fat. This may be a sign of commencement of fatty degeneration of the cell as suggested by Corner (1915) and others. It may also be stated that the appearance of these large vacuoles in the cell is accompanied by degenerative changes in the nucleus of such cells.

Another element of histological interest in the luteal cells of *Rhinobatus granulatus* is the chromatic granules. These are best shown in corrosive sublimate fixed material stained with iron hæmatoxylin. They are fine in the earlier stages but appear to grow larger and more apparent. Cesa-Bianchi (1908) describes similar granules densely crowded about the nucleus. The presence of such chromatic granules, has also been demonstrated by Corner (1915), scattered diffusely through the endoplasm. Regarding the nature of these granules Cesa-Bianchi (1908) states that since they are not dissolved by ether, alcohol or water from the fixed material and are preserved by mercury salts they are of an albuminous nature. Corner (1915) also supports this view. The chromatic granules in the cytoplasm of luteal cells of *Rhinobatus* are very probably of the same nature.

Theca interna.—The discussion of the origin of the luteal cells is inseparable from the question regarding the role the theca interna plays which is still a matter of controversy. The histological changes undergone by the theca interna cells in this case are very interesting. As has been described the cells of the theca interna undergo a unique process of development arranging themselves round a clear space forming tubule-like structures. Both in size and cytological structure the cells constituting these tubules are different from the follicular epithelial cells in the early stages of the formation of the corpus luteum. The nuclei are oval and the cell limits are ill defined. During the transformation of these cells into luteal elements they swell up and the cell-membrane becomes distinctly marked. The nucleus gradually assumes a vesicular form, the chromatin granules concentrating in a uniform manner at the inner surface of the nuclear wall. With the assumption of luteal condition the nucleolus becomes a prominent feature of the nucleus. When the cell reaches its maximum growth the whole cytoplasm is honey-combed with regular ring-like vacuoles as in the epithelial luteal cells. They also show the presence of chromatic granules. Consequently in the fully developed corpus luteum there is no difference in size or cytological structure between the epithelial luteal cells and the theca luteal cells.

With regard to the fate of the theca interna cells Van der Stricht (1912) considers that the theca interna of the follicle incorporates interstitial cells which become indistinguishable from the true lutein cells developed from the follicular epithelium and function as lutein cells in the fully developed corpus luteum. Corner (1915) has shown in the swine, that the theca interna cells take an important part in the constitution of the fully developed corpus luteum and some of the cells of the theca interna " come to resemble the true lutein cells very closely; indeed, at times it is almost impossible to distinguish them ". Novak's (1921) figures of the corpus luteum in his book *Human Menstruation and Its Disorders*, are in agreement with those of Corner's in the Sow. In a more recent paper Corner (1919) states that the theca interna cells may approximate very closely to the luteal cells and there is a confusing resemblance between some of the theca cells and follicular luteal cells. However, Corner distinguishes the theca interna cells from the luteal cells by the presence of vacuoles and rings in the luteal cells in his latest results (1921). It may be noted that there is no such difference between the two luteal elements of *Rhinobatus*. It may be summarised that according to the above authors the theca interna cells give origin to glandular cells showing striking resemblance to the epithelial luteal cell. This is in close agreement with the results of the present study.

Gatenby (1924) working on the human corpus luteum found clumps of small luteal cells situated near the theca externa which pass imperceptibly into larger luteal cells. Gatenby puts forward two views regarding the question of the origin of the luteal cells and the fate of the theca interna cells, one, " that the luteal cells are of mixed origin; that most of the theca interna cells so closely approximate in cytological arrangement to the membrane cells that it is impossible to distinguish the two; and finally that the stellate cells are fibroid elements derived from free cells of the theca externa", and the second which the author seems to favour "to look upon the stellate cells as theca interna elements, and thus to deny the theory of mixed origin of lutein cells ". The author, however, concludes that before more material has been studied it is not possible to draw a definite conclusion regarding the stellate elements. He further observes that it is difficult to deny the mixed origin of luteal cells because of the possibility that the follicular epithelial cells and thecal cells which are both derived originally from the mesoderm could undergo the same kind of specialisation and differentiation.

Apart from the theca interna cells which transform into luteal cells, another category of thecal cells has been described interspersed between the luteal cells in the fully developed corpus luteum. In the early stages these occur as groups of free thecal cells situated in the periphery of the ruptured

follicles wedged in between the concentric layers of the theca externa. Later on, these cells invade the theca interna along with the ingrowths of protoplasmic cells of the theca externa and lie in between the tubules of the theca interna. Later still a small number grow along with connective tissue fibres in among the diffuse groups of follicular luteal cells. In the fully developed corpus luteum these cells are found along the connective tissue fibres which penetrate in between the groups of luteal cells and also fit into the interspaces between the groups of luteal cells. In the fully developed condition they are spherical or oval with deeply staining vesicular nuclei. The cytoplasm is packed with minute regular vacuoles as in the luteal cells but the chromatic granules are not noticed. They at no time approximate to the luteal cells in size. These cells form a special group of cells different in histological character, location and origin from the luteal cells. In the corpus luteum of Sow, Corner (1915) describes two kinds of cells apart from the luteal cells, and denotes them as additional cells of the corpus luteum, type 1 and type 2. These free thecal cells seem to resemble in certain respects additional cell type 1 of Corner's description but there are no elements corresponding to type 2 of Corner.

Theca externa.—There is considerable variation in the behaviour of the theca externa in different animals. According to Sobotta (1896 and 1897) in the mouse and rabbit the theca externa does not take any part in the formation of the corpus luteum and the connective tissue ingrowth takes place exclusively from the theca interna. Marshall (1904) states that the connective tissue element of the corpus luteum in sheep is contributed both by the theca interna and theca externa. In the human corpus luteum Gatenby (1924) describes that the connective tissue ingrowths in the form of columns and lamellæ carrying blood vessels take place from the theca externa while small cells probably derived from the theca interna form the general supporting tissue. In *R. granulatus* the theca externa early gives rise to connective tissue ingrowths which carry blood capillaries and free thecal cells. In the later stages there is an extensive invasion of connective tissue elements of the theca externa in among the luteal tissue carrying blood vessels in the same manner as is observed in mammals and certain reptiles. The theca externa forms the enveloping sheath and also provides a delicate investment around the groups of luteal cells in the fully mature corpus luteum.

The present investigation shows that the corpus luteum in *R. granulatus* is a well-developed glandular organ exhibiting a close resemblance to the mammalian corpus luteum but showing noteworthy peculiarities of its own. As in the mammals the cavity of the discharged follicle is eventually filled

in by the luteal cells and ingrowth of connective tissue cells and fibres. It has also been possible to confirm the mixed origin of the luteal cells as it is shown that they are derived not only from the follicular epithelium, but a fair proportion of them is developed out of the cells of the theca interna.

Summary

1. A detailed description of the histology and development of the corpus luteum in *R. granulatus* from a very early stage upto the formation of a solid glandular body is given.

2. All the three elements of the follicular wall of the ruptured Graffian follicle, *viz.*, follicular epithelium, theca interna and theca externa take part in the final formation of the corpus luteum.

3. The luteal cells are shown to be formed by the hypertrophy of the cells of the follicular epithelium and the theca interna. The mixed origin of the luteal cells is described in a lower vertebrate for the first time.

4. The histological differentiation which takes place in the cells of the theca interna during their transformation into luteal cells is described in detail.

5. The theca externa forms both the enveloping sheath and the supporting framework of the fully developed corpus luteum. There is an extensive invasion of connective tissue elements of the theca externa carrying blood vessels and free thecal cells into the luteal tissue.

6. It has also been shown that degenerative changes set in very late. The close analogy between the formation of the corpus luteum in the Elasmobranchs and the mammals is pointed out.

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REFERENCES

Belloy* .. "Recherches sur l'origine des corps Jaune de l'ovaire chez le rat et le cochon d' Inde," *C. R. de l' Assoc. des Anatomistes* Premierè Session, Paris, 1899.

Bischoff, T. L. W.* .. "Entwickelungsgeschichte des Kaninchenes," *Braunschweig* 1842.

Buhler, A.* .. "Entwickelungsstudien menschlicher corpora lutea," *Verhand. d. Anat. Gesellschaft. in Pavia*, 1900.

———— .. "Ruckbildung der Eifollikel bei wirbelthieren," *Morph. Jahrb.*, 1902, **30.**

Cesa-Bianchi, D.* .. "Di alcune particolarita di strutturi edeifenomeni di secrezione del corpo luteo," *Internat. Monatschr. f. Anat. u. Physiol., Leipzig*, 1908, **25.**

Clark, J. G.* .. "The origin, growth, and fate of the corpus luteum as observed in the ovary of the pig and man," *Johns Hopkins Hosp. Rep., Baltimore*, 1898, **7.**

Cohn, F. .. "Zur Histologie und Histogenesis des Corpus Luteum und des Interstitiellen Ovarialgewebes," *Arch. f. Mikr. Anat.*, 1903, **62.**

Corner, G. W. .. "The corpus luteum of pregnancy, as it is in swine," *Contributions to Embryology*, 1915, **2,** Carnegie Institution of Washington.

————* .. "On the origin of the corpus luteum of the Sow from both granulossa and theca interna," *Amer. Jour. Anat.*, 1919, **26.**

———— .. "Cyclic changes in the ovaries and uterus of the Sow, and their relation to the mechanism of implantation," *Contributions to Embryology*, 1921, **13,** Carnegie Institution of Washington.

Cunningham, J. T. .. "On the histology of the ovary and of the ovarian ova in certain Marine fishes," *Q. J. M. S.*, 1898, **40.**

Deanesly, R. .. "The corpora lutea of the mouse with special reference to Fat accumulation during the Oestrous cycle," *Proc. Roy. Soc. Lond.*, Ser. B, 1930 *a*, **106.**

———— .. "The development and vascularisation of the corpus luteum in the mouse and rabbit," *ibid.*, Ser. B, 1930 *b*, **107.**

Doering .. "Beitrag zur streitfrage über die Bildung des Corpus Luteum," *Anat. Anz.*, 1899, **16.**

Gatenby, J. B. .. "Notes on the Human ovary, with special reference to a corpus luteum of ovulation," *Proc. of the Roy. Irish Academy*, 1921–24, **36.**

Giacomini* .. "Contributo all, 'Istologia' dell' Ovario dei Selaci", *Ricerca laboratoria di, Anatomia normale della Roy. Universita di Roma*," 1896, **5.**

Heape, W. .. "The menstruation and ovulation of *Macacus rhesus*, with observations on the changes undergone by the discharged follicle, *Phil. Trans. Roy. Soc. Lond.*, Ser. B, 1897, **188.**

Hill, J. P., and Gatenby, J. B. .. "The corpus luteum of the Monotremata," *Proc. Zool. Soc., Lond.*, 1926, 715–63.

His* .. "Discussion," *Verhand. d. Anat. Gesell., in Tübingen*, 1899.

Honore, C. H. .. "Recherches sur l'ovaire du lapin. I. Note sur les corps de call et exner et la formation du liquor folliculi. II. Recherches sur la formation des corpus jaunes," *Archives de Biologie, Tome*, 1900, **16.**

——— .. "Recherches sur l'ovaire du Lapin. III. Notes sur des follicules de. Graaf a'plusieurs ovules," *ibid.*, 1900, **17.**

Jankowski .. "Beitrag zur Entstehung des corpus luteum der säugethiere," *Arch. f. mikr. Anat.*, 1904, **44.**

Loeb, L.* .. "Über die Entwicklung des corpus luteum beim Meer schweinchen," *Anat. Anz.*, 1906, **28.**

Marsball, F. H. A. .. "The œstrous cycle and the formation of the corpus luteum in sheep," *Phil. Trans. Roy. Soc. Lond.*, Ser. B, 1904, **196.**

——— .. "The development of the corpus luteum"—A Review, *Q. J. M. S.*, 1906, **49.**

——— .. *The Physiology of Reproduction*, 1922.

Meyer, R.* .. "Über corpus luteum Bildung bein Menschen," *Arch. f. Gynæk, Berlin*, 1911, **93.**

Miller* .. "Corpus luteum, Menstruation und Graviditat," *ibid.*, 1914, **101.**

Nagel* .. "Uber neure Arbeiten auf dem Gebiete der Anatomie der weiblichen Geschlechtsorgane," Merkel and Bonnet's *Ergebnisse d. Anat. u. Entwickelungs*, 1899, **8.**

Novak, E.* .. *Menstruation and its Disorders*, Appleton, 1921.

Paterson* .. "Observations on corpora lutea," *Edinburgh Med. and Surg. Jour.*, 1840.

O'Donoghue, C. H. .. "The growth-changes in the Mammary apparatus of Dasyurus and the relation of the corpora lutea thereto," *Q. J. M. S.*, 1911, **57.**

——— .. "The corpus luteum in the non-pregnant Dasyurus and polyovular follicles in Dasyurus," *Anat. Anzeig.*, 1912, **B 41.**

——— .. "Über die corpora lutea bei einigen Beuteltieren," *Archiv. f. mikros. Anat.*, 1914, **Bd. 84.**

——— .. "On the corpora lutea and Interstitial tissue of the ovary in the Marsupialia," *Q. J. M. S.*, 1916, **61.**

Paladino .. "Per la Dibuttata Questione sulla Esenza del corpo luteo," *Anat. Anz.*, 1900, **18.**

Rabl, H.* .. "Beitrag zur Histologie des Eierstocks des Menschen und der säugetiere, nebst Bemerkungen über die Bildung von Hyalin und pigment," *Anat. Heft, I. Abt., Wiesbaden* 1898, **11.**

Robinson .. "The formation, rupture and closure of ovarian follicles in Ferrets," *Trans. Roy. Soc. Edin.*, 1918, **52.**

Sandes, F. P. .. "The corpus luteum of *Dasyurus viverrinus*, with observations on the growth and atrophy of the graafian follicle," *Proc. Linn. Soc. N.S.W.*, 1903.

Schrön, O.* .. "Beitrag zur kenntniss der Anatomie und physiologie des Eierstocks der Säugethiere," *Zeitschr. f. wiss. Zool. Leipzig*, 1863, **12.**

Sobotta, J. .. "Über die Bildung des corpus luteum bei der Maus," *Arch f. mikros. Anat.*, 1896, **Bd. 47.**

Stratz* .. *Der Geschlechtsreife Säugethiereierstock*, Haag, 1898.

Van der Stricht, O.* .. "La Rupture du Follicule ovarique et l'Histogénèse du cor jaune," *C. R. De l'Assoc. des Anatomistes*, 1901.

———— .. "Sur le processus de l'excretion des glandes endocrines *Arch. de Biol.*, 1912, **27.**

Von Baer, C. E.* .. "De ovi mammalium et Hominis Genesi, Epistola" 4°, *Lips L. voss.*, 1827.

Wallace, W. .. "Observations on ovarian ova and follicles in certain Tele stean and Elasmobranch Fishes," *Q. J. M. S.*, 1904, **47.**

Williams* .. *Obstetrics*, New York and London, 1904.

* Have not been referred to in original.

EXPLANATION OF PHOTOMICROGRAPHS

Rhinobatus granulatus Cuv.

PLATE VI

PHOTOMICROGRAPH 1. T.S. of a fairly ripe egg showing the structure of the follicular w ×200.

„ 2. T.S. of the follicular wall of the fully matured egg showing the tubul like structures of the theca interna. ×200.

„ 3. T.S. of the corpus luteum (Stage I). ×48.

„ 4. T.S. of the corpus luteum (Stage I); a portion magnified to show t follicular epithelial folds, the tubules of theca interna and the the externa. ×80.

„ 5. Follicular epithelium (Stage I), magnified to show the highly hype trophied follicular epithelial luteal cells. ×400.

„ 6. A portion of the theca interna (Stage I) magnified to show the cells of t tubules of theca interna, the connective tissue fibres and the fre thecal cells. × 400.

PLATE VII

PHOTOMICROGRAPH 7. T.S. of the corpus luteum (Stage III). ×48.

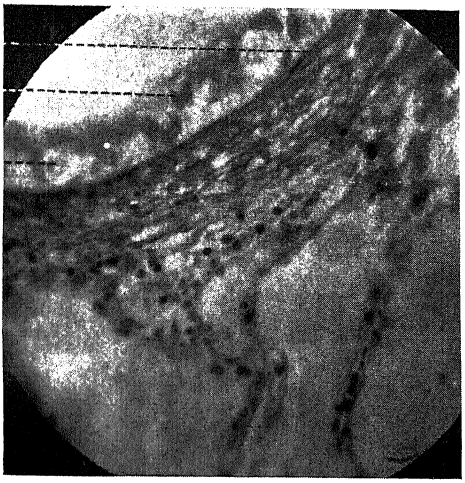
„ 8. A magnified view of the follicular epithelial luteal cells (Stage III). ×40

„ 9. A portion of the theca interna (Stage III) magnified to show the tran forming cells of the theca interna. ×400.

„ 10. T.S. of the corpus luteum (Stage IV) showing the ingrowth of the prot plasmic cells of the theca externa. ×48.

„ 11. T.S. of the corpus luteum (Stage IV); a portion of the wall of the corp luteum magnified to show the tubules of theca interna and t follicular epithelial luteal cells. ×80.

„ 12. Magnified view of the follicular epithelial luteal cells (Stage IV). ×40



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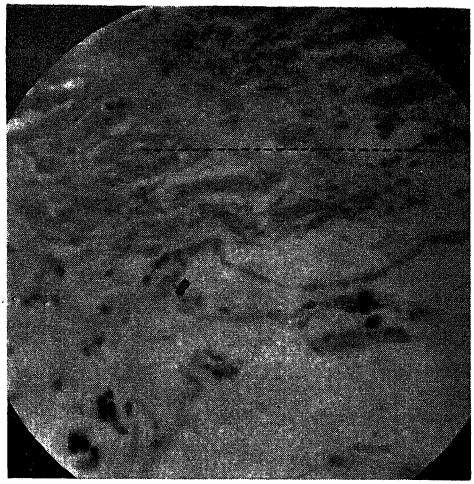

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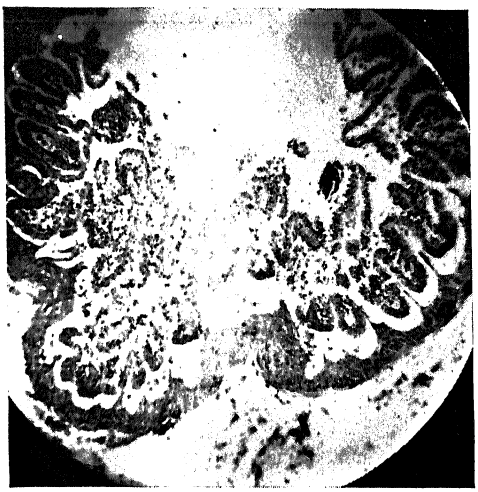

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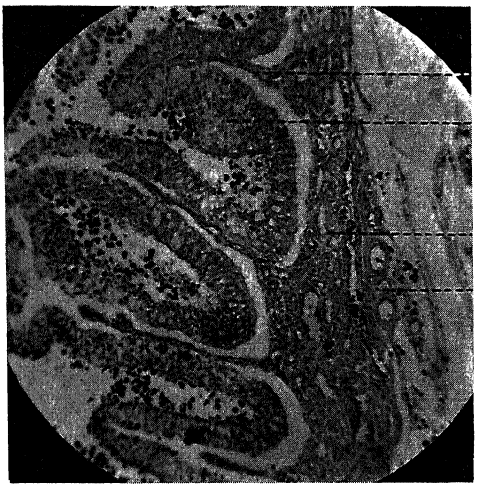

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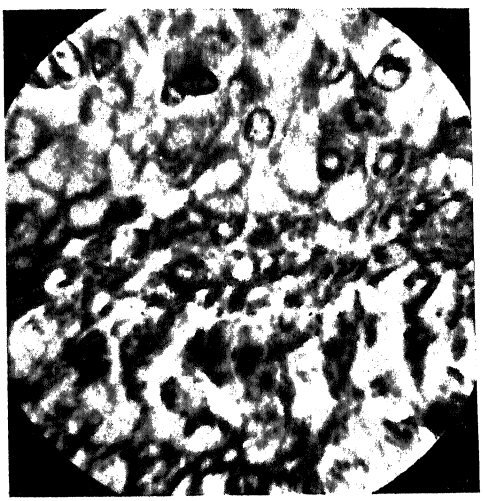

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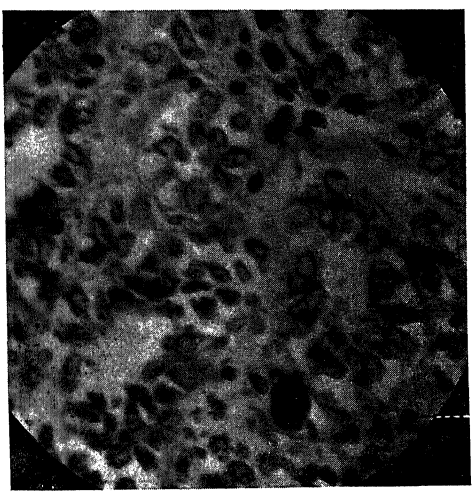

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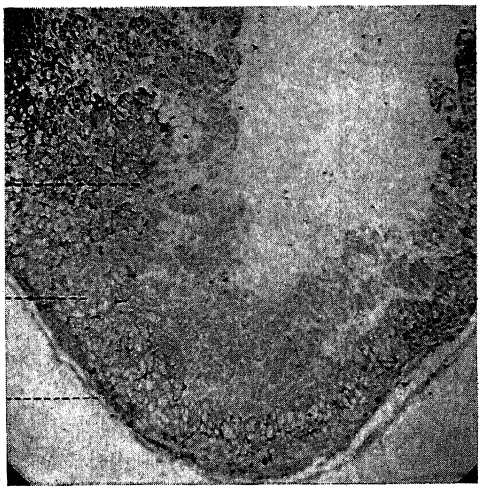

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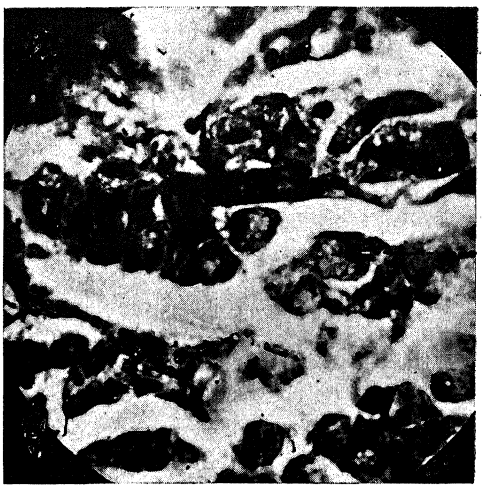

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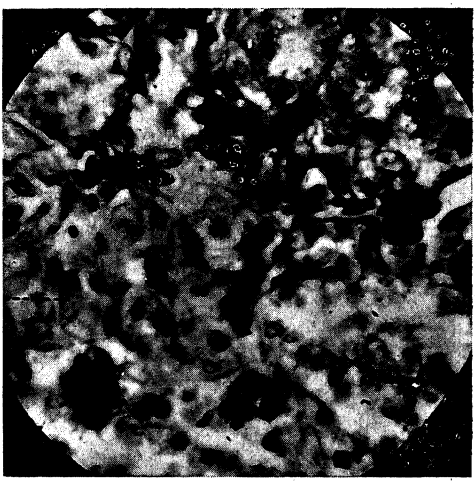

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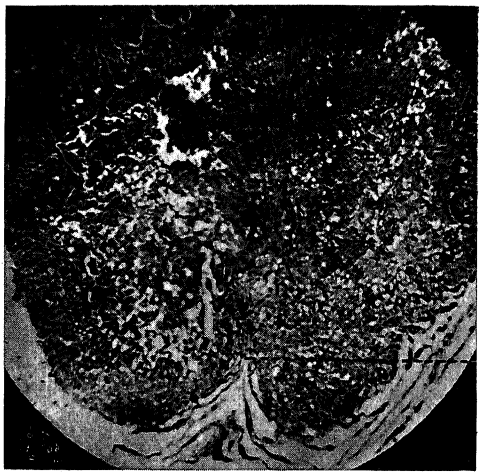

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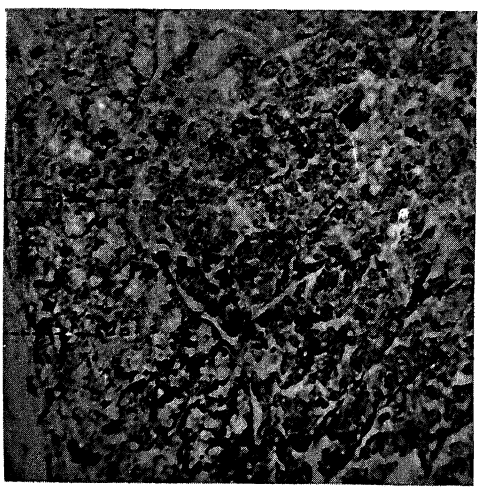

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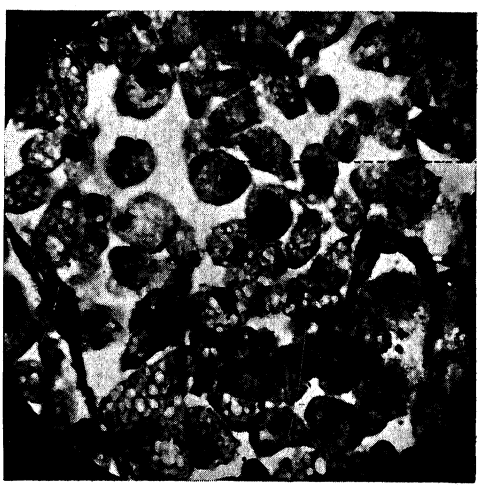

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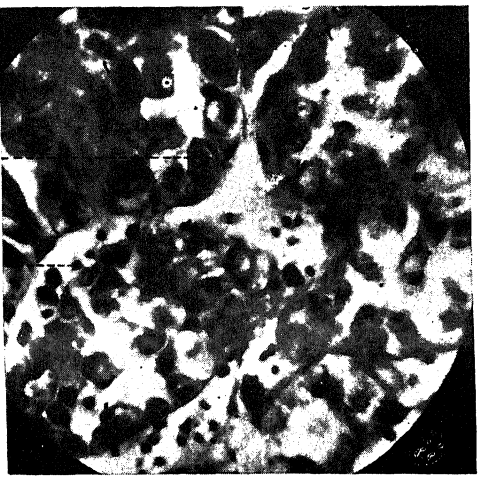

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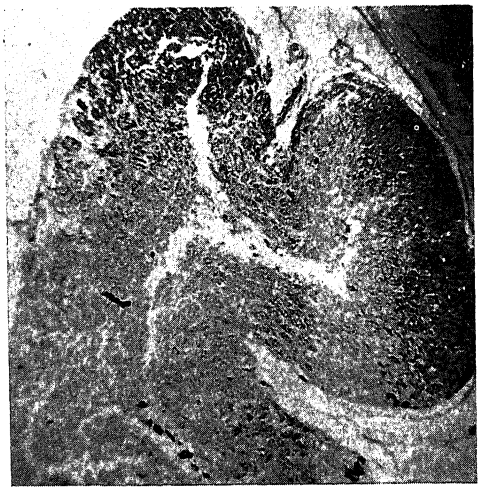

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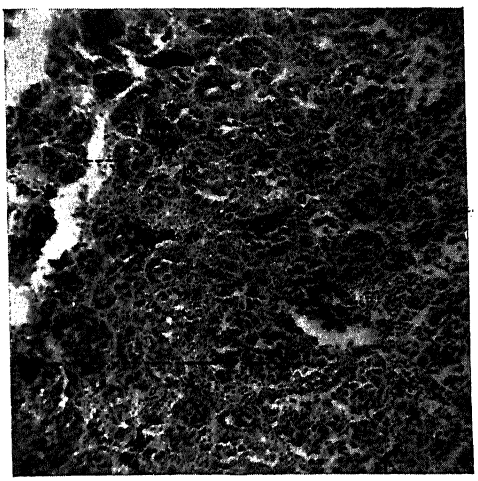

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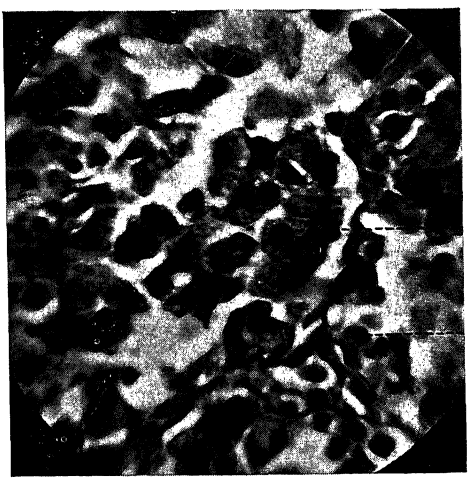

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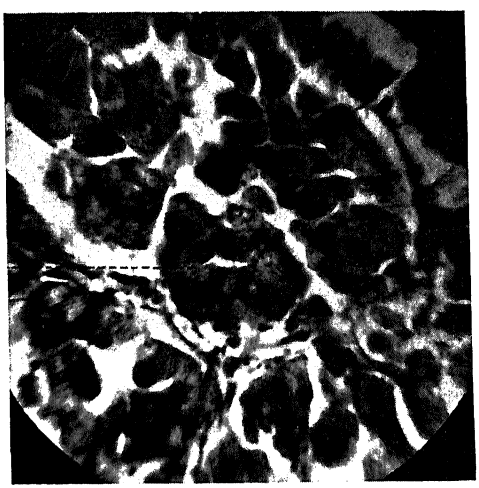

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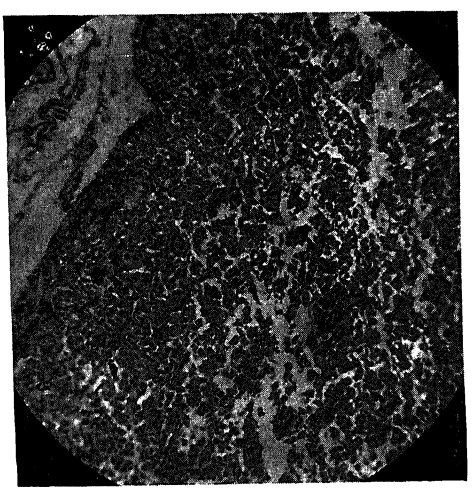

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PLATE VIII

PHOTOMICROGRAPH 13. A portion of the theca interna (Stage IV) magnified to show the transforming theca interna cells and free thecal cells. ×400.

„ 14. T. S. of the fully developed corpus luteum (Stage V). ×48.

„ 15. T. S. of the corpus luteum (Stage V); a portion magnified to show the tubules of theca interna luteal cells and follicular epithelial luteal cells. ×80.

„ 16. A magnified view of a group of fully developed follicular epithelial luteal cells, the free thecal cells and the connective tissue fibres in between the groups of luteal cells (Stage V). ×400.

„ 17. Theca interna luteal cells (Stage V) magnified to show the similarity between the theca interna luteal cells and follicular epithelial luteal cells. ×400.

„ 18. T. S. of the solid corpus luteum (Stage VI) showing the general rupture of the tubules. ×80.

N.B.—All figures have been drawn with the Camera lucida. Text-Figs. have been reduced to half and Photomicrographs by one-eighth the magnifications given.

KEY TO LETTERING

b.ca. .. Blood capillaries.
b.v. .. Blood vessel.
c.tu.th.i. .. Cells of the tubules of theca interna.
ch.g. .. Chromatic granules of luteal cells.
cn.t.fi. .. Connective tissue fibres.
cn.t.ig. .. Connective tissue ingrowths.
d.l.c. .. Degenerating luteal cell.
en. .. Endothelium.
f.e. .. Follicular epithelium.
f.e.c. .. Follicular epithelial cell.
f.e.fo. .. Follicular epithelial fold.
f.e.l.c. .. Follicular epithelial luteal cell.
f.e.fo. .. Follicular epithelial fold.
fg.th.i. .. Finger-like ingrowths of the theca interna.
fr.th.c. .. Free thecal cell.
ho.l.c. .. Homogeneous staining area of the cytoplasm of the luteal cells.
ig.th.ex. .. Ingrowths of protoplasmic cells of theca externa.
l.c. .. Luteal cells.
la.c. .. Large cell of follicular epithelium.
m.p. .. Membrana propria.
s.c. .. Small cell of the follicular epithelium.
sm.va. .. Small regular vacuoles of luteal cells.
th.ex. .. Theca externa.
th.fl. .. Theca folliculi.
th.i. .. Theca interna.
th.i.l.c. .. Theca interna luteal cell.
tr.th.i.c. .. Transforming theca interna cell.
tu.th.i. .. Tubules of theca interna.
z.r. .. Zona radiata.

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